

Chemical Characterization and Application of the Essential Oils from *Chenopodium ambrosioides* and *Philodendron bipinnatifidum* in the Control of *Diabrotica speciosa* (Coleoptera: Chrysomelidae)

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Abstract

The compositions of essential oils from *Chenopodium ambrosioides* L. and *Philodendron bipinnatifidum* Schott were determined, and their potential effects on the nutrition and mortality of *Diabrotica speciosa* were studied. The extraction of the oils was performed by hydrodistillation (2 h) using a modified Clevenger apparatus and the oils were subsequently subjected to analysis by gas chromatography/flame ionization detector (CG/FID) and gas chromatography/mass spectrometry (GC-MS). A completely randomized design with five treatments and four replications was adopted. The bean plants were sprayed with solutions of the oils dissolved in aqueous Tween 80 solutions at concentrations of 0 (water + Tween 80), 0.5%, 1.0%, 1.5% and 2.0% and then furnished to the insects with no choice available. Seven days after the application, the percentage of leaves with injury, degree of defoliation, the preference index for consumption and the percent of mortality of insects were evaluated. Neither of the essential oils caused a reduction in foliar injury, but anti-feeding activity was observed, causing reduced feeding and increasing the mortality of adult *D.*

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speciosa insects.

Keywords

Dietary Behavior, Insecticidal Activity, Bean, Natural Products

1. Introduction

Diabrotica speciosa (Germar, 1824) (Coleoptera: Chrysomelidae) is a major pest of dry beans and some vegetable crops. It has spread throughout all the Brazilian states and other countries of South America [1]. In Brazil, in addition to beans [2], this pest has been recorded to cause damage to corn [3], soy [4], and potatoes [5], among others.

The control of this insect pest is accomplished primarily by spraying synthetic insecticides based on active ingredients such as lufenuron [6], β -cyfluthrin and methamidophos [7]. But, the development of insect resistance to these products, the high operational cost and environmental impacts have created the need for developing alternative approaches to control many insect pests, and in this sense the essential oils are an alternative to control many insects [8].

Essential oils are complex mixtures of volatile compounds of different chemical origins [9]. They possess important biological activities, especially for the agrochemical industry that seeks to develop natural insecticides. In this context, these compounds are an alternative for the control of insect pests [10]. They may act as repellants [11], reduce feeding activity [12] or cause death [13].

Chenopodium ambrosioides L., popularly known as Santa Maria herb, is a native species of tropical South America, principally Mexico. Currently, it is distributed throughout the tropical, subtropical and temperate regions. The essential oil from *C. ambrosioides* contains substances with fungicide, acaricide, bactericide, nematocidal, insecticide, molluscicide and allelopathic properties [14].

The *Philodendron bipinnatifidum* Schott (Araceae) species is widely distributed throughout Brazil. It has root struts by which it rests on a support and roots for absorption of water and minerals [15]. The roots of different species of *Philodendron* are used in traditional medicine in the Amazon region, as well as for bathing and fumigation [16].

The study of plants with insecticidal activity to provide data that serve to aid in the development of insecticides of plant origin that have interesting biological potentials is important. The importance of studying the *C. ambrosioides* L. Schott and *P. bipinnatifidum* species should be emphasized. The present study involved the characterization of the essential oils from these two species and the evaluation of their effects on foliar injury, feeding activity and mortality of *D. speciosa* in the common bean plant.

2. Material and Methods

2.1. Extraction of Essential Oils

The leaves of the *C. ambrosioides* plant were collected in the Medicinal Plants Garden of the Federal University of Lavras (UFLA), Minas Gerais (MG), Brazil. The roots of the *P. bipinnatifidum* species were collected on a farm in the municipality of Pains, MG, Brazil. The collection site for *C. ambrosioides* has the following coordinates: 21°13'49.0476"S, 44°58'27.4764"W and 933 m altitude. The location for the collection of *P. bipinnatifidum* was 20°22'13"S, 45°65'71"W and 923 m altitude. The ratification of the taxonomic species was performed in the ESAL Herbarium (Herbarium, Department of Biology, UFLA). A voucher specimen of each species was incorporated into the collection of the Herbarium under registration number ESAL 26769 for *C. ambrosioides* and registration number ESAL 27111 for *P. bipinnatifidum*.

The extraction of the essential oils was performed at the Laboratory of Organic Chemistry—Essential Oils, Department of Chemistry, UFLA. The material was subjected to hydrodistillation for two hours using a modified Clevenger apparatus coupled to a 5-liter round bottom flask. The hydrolact was collected and centrifuged in a horizontal crosshead centrifuge at 1100 g for 5 min. The essential oil was removed with the aid of a Pasteur pipette, packed in a glass flask that was wrapped with aluminum foil and stored under refrigeration [17].

2.2. Identification of Constituents of the Essential Oils

The GC-MS analyses were performed on a Perkin Elmer Autosystem XL gas chromatograph equipped with a fused silica column (30 m × 0.25 mm ID, DB-1 film thickness, 0.25 μm; J & W Scientific Inc.) coupled to a Perkin Elmer TurboMass mass spectrometer (software version 4.1). The oven temperature was programmed from 45°C to 175°C at a rate of 3°C/min, and, subsequently, 15°C/min to 300°C, where the temperature was held constant for 10 min. The transfer line temperature was 280°C, the temperature of the ionization chamber was 220°C, and the carrier gas was helium at a linear velocity of 30 cm/s. The split ratio was 1:40.

The identities of the compounds were determined by comparison of their retention indices with those of the C9-C21 n-alkanes and by comparing the mass spectra with those of standard commercial and reference compounds present in existing oils in the laboratory, as well as by comparison with a mass spectral library developed at the laboratory of the Centro de Biotecnologia Vegetal, Faculdade de Ciências, Universidade de Lisboa [18].

2.3. Quantification of Constituents of Essential Oils

The essential oils were analyzed by gas-liquid chromatography on a Perkin Elmer 8700 gas chromatograph equipped with two flame ionization detectors, a system for processing data and an autoinjector. Two columns of different polarity were installed with the following characteristics: DB-1 methyl silicone immobilized phase in a fused silica column (30 m × 0.25 mm ID, film thickness 0.25 μm; J & W Scientific Inc.); DB-17HT phenylmethylsilicone stationary phase (30 m × 0.25 mm ID; film thickness 0.25 μm). The oven temperature was programmed from 45°C to 175°C at a rate of 3°C/min, and, subsequently, 15°C/min to 300°C, where the temperature was maintained for 10 min. The temperature of the injector and detector ports was 290°C and 280°C respectively. The carrier gas was hydrogen, adjusted to a linear velocity of 30 cm/s. The split ratio was 1:50. The percentage composition of the oils was determined by integration of peak areas without using correction factors. The values given represent the average of two injections [18].

2.4. Bioactivity of Essential Oils

Bioassays with *D. speciosa* were conducted at the Laboratory of Plant Resistance to Insects, Department of Entomology, UFPA, according to the method described by Assis *et al.* [19], with modifications. The bean plants were grown in pots with a capacity of 3 kg using the C Horizon soil (Latossolo Vermelho Escuro) fertilized with 3 g of NPK fertilizer (8-28-16) per pot, equivalent to 450 kg·ha⁻¹, as substrate. Four carioca beans seeds were planted per vessel. The pots were kept on benches inside the greenhouse. Thinning was performed 20 days after planting, leaving three plants per pot.

The collection of non-sexed *D. speciosa* adults was performed in a corn field with the aid of a plastic aspirator attached to a glass container. Subsequently, the beetles were taken to the laboratory, where they were kept for 24 h in an acrylic cage (30 × 30 × 80 cm) and fed with bean plants grown in pots.

A completely randomized design (CRD) with five treatments and four replications was employed. The statistical program used was Sisvar. Data were subjected to analysis of variance, and the average was compared by the Scott Knott Test at 5% probability [20]. The bean plants were sprayed with solutions of essential oils dissolved in aqueous Tween 80 at concentrations of 0.5%, 1.0%, 1.5% and 2.0% using a 10 mL plastic hand sprayer. A 2.0% solution of Tween was applied as the control treatment. The solutions were sprayed to the point that they ran down the leaves and stems of the plants.

The vessels were distributed randomly on benches inside the climate chamber (temperature equal to 30°C during the day and 25°C at night, relative humidity, 70% ± 10% and 12 h of light) and covered with organza fabric supported on two iron rods fixed to the substrate of the vessel, forming a cage of approximately 20 cm diameter and 60 cm high. Ten adults per cage were released after treatment [19]. Seven days after the application of the essential oils, the percentage of leaves with injury and the degree of defoliation were evaluated using the AM-300 portable meter (ADC BioScientific Ltd, England). The percent of insect mortality was also determined. In addition, the preference index (PI) with respect to consumption was also calculated according the method of Goeden and Kogan [21] using the following formula: $PI = 2A/A + M$, where A = degree of defoliation (plants with application of essential oils), M = consumed leaf area (control plant), $PI = 1$ (neutral), $PI < 1$ (phagodeterrent), $PI > 1$ (phagostimulant).

3. Results and Discussion

3.1. Chemical Composition of the Essential Oils

The chemical components of essential oil of *C. ambrosioides* L. and *P. bipinnatifidum* followed by their calculated retention index and reported retention index with their contents expressed in percentage are in **Table 1** and **Table 2**, respectively.

The principal component found in the essential oil from *C. ambrosioides* was α -terpinene (40.7%), followed by *p*-cymene (21.8%) and *trans*-ascaridol (12.5%) (**Figure 1**). A predominance of monoterpene hydrocarbons (62.8%) and oxygenated monoterpenes (13.2%) was observed.

The data obtained in this study confirm those of Monzote *et al.* [22], who identified α -terpinene, *p*-cymene and ascaridol as the principal constituents of the oil from *C. ambrosioides*, with variations in chemical composition from 17.0% to 20.7%, from 20.2% to 21.1% and from 30.5% to 47.1%, respectively. Moreover, Borges *et al.* [23] encountered terpinolene (69.9%) and ascaridol (17.1%) in the essential oil from this species collected in Brazil. However, α -terpinene (51.3%), *p*-cymene (23.4%) and *p*-mentha-1,8-diene (15.3%) were the principal compounds encountered in *C. ambrosioides* plants harvested in Cameroon (Africa). This difference can be explained by the different locations where the plants were collected, among other factors [24].

The essential oil from *P. bipinnatifidum* contained β -bisabolene (65.3%), *trans*- α -bergamotene (9.9%) (**Figure 2**), being composed principally of the sesquiterpene hydrocarbon compounds (91.6%) and monoterpene hydrocarbons (3.7%) (**Table 2**). Although the literature reports on the yield and composition of the essential oil from the roots of *P. bipinnatifidum* are still scarce, α -pinene (13.3%), β -pinene (15.8%), limonene (15.5%), spathulenol (14.2%) and caryophyllene oxide (10.3%) have been found in the essential oils of plants of the *Philodendron* genus [25]. This composition differs from the data presented in the present study. The discrepancy of this information is probably related to the fact that the species studied are distinct and have a resemblance only at the genus level.

Table 1. Composition of the essential oil from *Chenopodium ambrosioides* L.

Compound	RI _{cal}	Percent Composition (%)
Benzaldehyde	927	v
α -Pinene	930	v
n-Octanal	973	v
β -Myrcene	975	v
α -Terpinene	1002	40.73
<i>p</i> -Cymene	1003	21.81
β -Phellandrene	1005	v
Limonene	1009	0.24
<i>trans</i> - β -Ocimene	1027	v
γ -Terpinene	1035	v
n-Octanol	1045	v
Dimethyl styrene	1059	v
n-Nonanal	1073	v
<i>cis</i> -Piperitone Epoxide	1211	0.34
<i>trans</i> -Piperitone Epoxide	1258	0.35
<i>trans</i> -Ascaridol	-	12.49
Total Identified		75.95%

RI_{cal} = Calculated retention index, N. area = Normalization of the area, v = vestigial (less than 0.01%).

Table 2. Composition of the óleo essential from *Philodendron bipinnatifidum* Schott.

Compound	RI _{cal}	Percent Composition (%)
α -Pinene	930	1.48
Canphene	938	0.12
Sabinene	958	0.17
β -Pinene	963	0.18
β -Myrcene	975	1.16
α -Phellandrene	995	v
α -Terpinene	1002	v
<i>p</i> -Cymene	1003	v
β -Phellandrene	1005	v
Limonene	1009	0.57
<i>cis</i> - β -Ocimene	1017	v
<i>trans</i> - β -Ocimene	1027	v
γ -Terpinene	1035	v
α -Cubebene	1345	0.20
Cyclosativene	1363	0.16
α -Ylangene	1371	0.03
α -Copaene	1375	3.33
7- <i>epi</i> - α -Cedrene	1396	0.23
α -Cedrene	1400	0.12
β -Caryophyllene	1414	0.95
<i>trans</i> - α -Bergamotene	1434	9.97
α -Himachalene	1441	0.16
α -Humulene	1447	0.20
<i>trans</i> - β -Farnesene	1455	2.30
β -Santalene	-	1.25
<i>trans</i> -Cadina-1(6),4-diene	1469	0.27
α -Curcumene	1475	1.99
β -Selinene	1476	0.73
<i>cis</i> - β -Guaiene	1478	0.05
Valencene	1484	0.07
γ -Muurolene	1493	0.54
α -Muurolene	1494	0.45
β -Bisabolene	1500	65.26
<i>trans</i> -Calamenene	1505	v
δ -Cadinene	1505	2.83
α -Calacorene	1525	0.28
Germacrene B	1533	0.12
<i>trans</i> - α -Bisabolene	1536	0.14
<i>trans</i> -Nerolidol	1549	v
α -Caryophyllene oxide	1561	0.05
1- <i>epi</i> -Cubenol	1600	0.17
<i>epi</i> - α -Cadinol	1616	v
β -Bisabolol	-	0.19
α -Bisabolol	1656	0.03
<i>epi</i> - α -Bisabolol	1658	0.06
Total Identified		96.51%

RI_{cal} = Calculated retention index, N. area = Normalization of the area, v = vestigial (less than 0.01%).

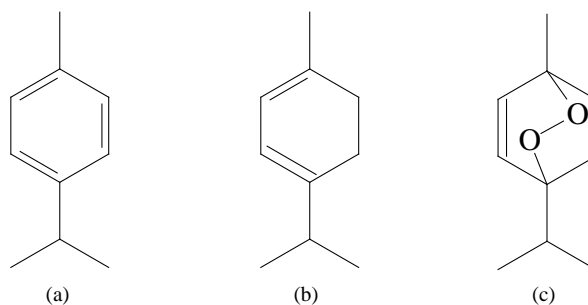


Figure 1. Chemical structures of the major components of the essential oil from *C. ambrosioides* L.: (a) *p*-cimene; (b) α -terpinene; (c) trans-ascaridol.

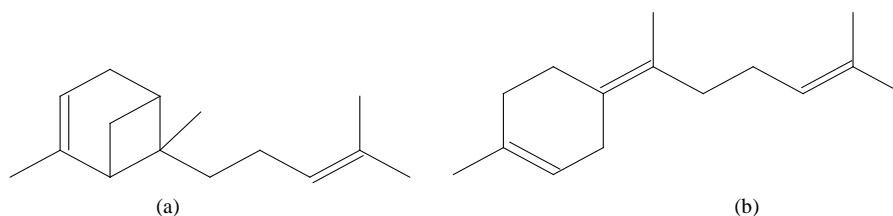


Figure 2. Chemical structures of the major components of the essential oil *P. bipinnatifidum* Schott: (a) trans- α -bergamotene; (b) β -bisabolene.

3.2. Activity of Essential Oils against *Diabrotica speciosa*

It appears from the data presented in **Table 3** that the use of the essential oils didn't contribute to a reduction in the number and degree of injuries in bean leaves caused by the chrysomelid because there was no significant difference between the treatments.

With regard to the leaf area consumed, an influence on the feeding behavior of the insect pest was observed for both the essential oils (**Table 4**). There was a decrease in the consumption of the leaves proportional to the increase in the concentrations of the essential oils.

With respect to the preference index for consumption, antifeedant activity ($PI < 1$) was observed for the essential oils at all the concentrations tested, *i.e.*, they inhibited feeding by the defoliator beetle (**Table 5**).

It is known that the eating behavior of insects depends on the integration of the central nervous system with the chemoreceptors located on the shanks, the mouth parts and the oral cavity and insecticides found in plants can act upon the chemoreceptors by stimulating deterrent cells or blocking phagostimulating cells to inhibit feeding [26]. The results are similar to those found by Seffrin *et al.* [27] regarding the use of natural products that exert antifeedant activity against *D. speciosa* on the common bean, although the plants studied are different from those of the present study.

The chromatographic analysis allowed the identification of terpenes in essential oils. According to Viegas - Júnior [28], the ecological importance of these compounds as pesticides plant is well elucidated, as several monoterpenes have been isolated and proved its toxicity against the different insects, ensuring a mortality ranging from 40% to 100%. In addition, other classes of terpenes have been researched for a better understanding of their suppressive activities of feeding and repellency.

It is important to note that the efficiency of extracts or essential oils can be variable, depending on the plant species used and the species of insect pest to be controlled. Although the present study involved another kind of coleoptera, the mortality observed can be compared with that observed for the maize weevil *Sitophilus zeamais* by Mots, 1855; Coleoptera; Curculionidae) using powders of fruits and the whole *C. ambrosioides*. It had a highly toxic effect on the beetle [29].

Moreover, the reduction in the survival of *D. speciosa* was proportional to the concentrations of the essential oils. There was a significant difference between treatments with regard to the percentage of insect mortality, proving that some insecticidal action of the essential oils existed (**Table 6**). The insecticidal activity of the essential oils against insects can be attributed to the principal chemical constituents. However, the occurrence of synergism with the minor components may also encourage this activity.

Table 3. Percentage of leaf injuries caused by *Diabrotica speciosa* in bean plants treated with different concentrations of the essential oils studied.

Species	Concentration (%)				
	0.0	0.5	1.0	1.5	2.0
<i>C. ambrosioides</i> L.	55.17 ± 13.48a	49.72 ± 10.18a	45.58 ± 10.20a	44.87 ± 5.75a	38.64 ± 6.15a
<i>P. bipinnatifidum</i>	58.20 ± 13.19a	62.53 ± 9.53a	86.46 ± 17.80a	86.83 ± 11.40a	72.30 ± 14.65a

Means followed by the same letter in the same line do not differ at a 5% significance level by the Scott Knott Test.

Table 4. Leaf area (cm²) consumed by *Diabrotica speciosa* on bean plants treated with different concentrations of the essential oils studied.

Species	Concentration (%)				
	0.0	0.5	1.0	1.5	2.0
<i>C. ambrosioides</i> L.	214.48 ± 25.52 a	172.70 ± 16.64ab	162.04 ± 53.17 ab	138.12 ± 19.28b	120.75 ± 31.48b
<i>P. bipinnatifidum</i>	532.78 ± 32.49a	298.39 ± 38.75 b	197.87 ± 25.76 bc	196.77 ± 39.21 bc	137.93 ± 26.71c

Means followed by the same letter in the same line do not differ at a 5% significance level by the Scott Knott Test.

Table 5. Preference index for feeding by *Diabrotica speciosa* on bean plants treated with different concentrations of the essential oils studied.

Concentrations (%)	Preference index	
	<i>C. ambrosioides</i>	<i>P. bipinnatifidum</i>
Control	1.00	1.00
0.5	0.55	0.64
1.0	0.58	0.73
1.5	0.61	0.73
2.0	0.64	0.80

PI = 1 (neutral); PI < 1 (phagodeterrent); PI > 1 (phagostimulant).

Table 6. Percent mortality of *Diabrotica speciosa* on bean plants treated with different concentrations of the essential oils studied.

Species	Concentration (%)				
	0.0	0.5	1.0	1.5	2.0
<i>C. ambrosioides</i> L.	0.00 ± 0.00b	5.00 ± 5.77b	15.00 ± 5.77ab	23.75 ± 9.46a	25.00 ± 19.14a
<i>P. bipinnatifidum</i>	0.00 ± 0.00b	2.50 ± 5.00b	12.00 ± 12.58ab	15.00 ± 12.91a	22.00 ± 18.93a

Means followed by the same letter in the same line do not differ at a 5% significance level by the Scott Knott Test.

Thus, the use of the essential oils from the leaves of *C. ambrosioides* and roots of the *P. bipinnatifidum* species can be a viable alternative for the control of *D. speciosa* in the common bean. However, field trials are needed to prove the efficacy of such oils in controlling this chrysomelid under natural conditions.

4. Conclusion

The essential oil from *C. ambrosioides* L. contains α -terpinene (40.73%), *p*-cymene (21.81%) and *trans*-ascaridol (12.48%) as the principal compounds. The essential oil from *P. bipinnatifidum* Schott contains β -bisabolene (65.26%) and *trans*- α -bergamotene (9.97%) as its major constituents. The application of the essential oils from

C. ambrosioides and *P. bipinnatifidum* affected the behavior of *D. speciosa*, reducing the feeding activity and causing the death of the insect pest.

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