CROP SCIENCE

Chemical composition and insecticidal activity of the essential oils of Piper marginatum, Piper callosum and Vitex agnus-castus

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Abstract: During grain storage, a considerable amount of product is lost because of insects, such as Zabrotes subfasciatus. Currently, to mitigate these risks, studies are searching for plants with potential for the control of agricultural pests, also known as botanical insecticides. In this study, the fumigant toxicity of the essential oils of Piper callosum (PC-EO), Piper marginatum (PM-EO) and Vitex agnus-castus (VA-EO) against Zabrotes subfasciatus was investigated. The essential oils of PC-EO, PM-EO and VA-EO were analysed by gas chromatography (GC-MS), and the major components were 3,4-methylenedioxypropiophenone (10.4%), bicyclogermacrene (10.1%) and germacrene D (9.9%) for PM-EO; safrol (29.3%) for PC-EO; and 1,8-cineol (23.8%) for VA-EO. In fumigation tests, VA-EO killed 100% Zabrotes subfasciatus at a concentration of 0.004 µL/L air after 24 h of treatment, whereas PC-EO and PM-EO at 0.01 µL/L air caused 100% Z. subfasciatus mortality after 48 h. The VA-EO sample provided the lowest LD50 after 24 h (0.17 µL/L air), followed by PC-EO (0.78 µL/L air) and PM-EO (1.17 µL/L air). These results demonstrate that the essential oils of these species can be an alternative to control pests in stored products. This is the first report of the fumigant potential of these species against Z. subfasciatus.

Key words: Botanical insecticides, Lamiaceae, Piperaceae, volatile compounds.

INTRODUCTION

The Amazon Region is very important worldwide because it has a rich, biodiverse ecosystem (Sawyer 2015). The essential oils of Amazonian aromatic plants are of great economic importance due to their various applications, such as flavourings, drug adjuvants, insect repellents, antimicrobials and antioxidants (Bizzo et al. 2009, Cook & Lanaras 2016). Among the aromatic plants present in the Amazon rainforest are Piper callosum Ruiz & Pav, Piper marginatum Jacq (Piperaceae) (Andrade et al. 2009) and Vitex agnus-castus L. (Lamiaceae) (Di Stasi & Hiruma-Lima 2002).

The essential oil of Piper callosum showed several biological activities, such as fungicide (Silva & Bastos 2007), antibacterial (Majolo et al. 2019), schistosomicidal (Gonçalves et al. 2019), larvicidal (Andrade et al. 2009), insecticide against Solenopis saevissima (Souto et al. 2011) and Bemicia tabaci (Fanela et al. 2015). The essential oil of Piper marginatum showed antioxidant activity (Bay-Hurtado et al. 2016), antibacterial (Majolo et al. 2019), schistosomicidal (Gonçalves et al. 2019), antileishmanial (Macedo et al. 2020), antiparasitic against Neochinorhynchus buttnerae (Santos et al. 2018), larvicidal against Aedes aegypti (Autran et al. 2009) and ovicidal potential against Anticarsia gemmatalis (Krinski
et al. 2018). Essential oil of Vitex agnus-castus has antimicrobial activity (Bakr et al. 2020, Gonçalves et al. 2017, Habbab et al. 2016, Eryigit et al. 2015, Stojković et al. 2011), antimitogenic (Sarac et al. 2015) and insecticidal against Bemisia tabaci (Fanela et al. 2015).

The essential oils of these medicinal species have not yet been evaluated for their insecticidal potential against Zabrotes subfasciatus (Boheman, 1833) (Coleoptera: Bruchidae), the main pest of the bean Phaseolus vulgaris L. This species is one of the most produced and consumed beans in Brazil and is considered an important protein source in the diet of Brazilians (Oliveira et al. 1979). However, during grain storage, a considerable amount of product is lost because of insects, such as Z. subfasciatus.

Several populations of insects are resistant to the currently registered synthetic insecticides (Pimentel et al. 2010, Boyer et al. 2012) that consist largely of phosphines for fumigation and pyrethroids and organophosphates for preventive control. In Brazil, phosphine and pyrethroids are allowed to control Zabrotes subfasciatus in stored beans seeds and grains, according to the Ministério da Agricultura, Pecuária e Abastecimento (2020). However, they are toxic and dangerous to the environment. Phosphine is very toxic to all forms of animal life, hence exposure of human beings even to small amounts should be avoided. The effectiveness of phosphine can be reduced considerably by development of resistance in insects (Bond 1984).

Currently, studies are searching for chemical compounds in plants with potential for the control of agricultural pests, also known as botanical insecticides (Mazzonetto & Vendramim 2003, Nakano et al. 1981). The advantages of botanical insecticides compared with conventional insecticides are related to their lower mammalian toxicity and decreased health risk to applicators and their rapid degradability, which reduces residues in the environment and in the treated products (Isman 2006). In this respect, essential oils have been shown to be potentially active as insecticides of plant origin against Z. subfasciatus. After 12-h treatment, essential oils extracted from Chenopodium ambrosioides L. and Ocimum gratissimum L. at 20.0 mL/L of air killed 100% Z. subfasciatus, whereas essential oil of Schinus terebinthifolius Raddi at 100.0 mL/L of air afforded 100% Z. subfasciatus mortality after 24 h (Bernardes et al. 2018). Essential oils of Citrus reticulata, Citrus medica limonum, Citrus sinensis, Copaifera langsdorffii, Baccharis dracunculifolia, Eucalyptus globulus, Eucalyptus citriodora, Cymbopogon citratus and Cymbopogon nardus significantly reduced viable egg-laying and the adult emergence of Z. subfasciatus, depending on the concentrations (França et al. 2012). Thus, the present research aims to characterize the chemical constituents of PM-EO, PC-EO and VA-EO and to evaluate their toxicity against Zabrotes subfasciatus.

MATERIALS AND METHODS

Plant material

*Piper marginatum* Jacq (Piperaceae) was collected at Highway AM 010 km 18 (S 03° 01’ 50.5” W 58° 32’ 37.3”), *Piper callosum* Ruiz & Pav (Piperaceae) was collected in Lake Serpa (S 03° 04’ 28.6” W 58° 28’ 36.3”), and *Vitex agnus-castus* L. (Lamiaceae) were collected near Itacoatiara (S 03° 08’ 28.8” W 58° 26’ 54.3”) in the state of Amazonas, Brazil. The species were identified by Prof. Ari de Freitas Hidalgo (Faculdade de Ciências Agrárias - UFAM). A voucher specimen of each plant was deposited in the Herbarium of the Universidade Federal do Amazonas (HUAM/
UFAM), under numbers 8266 (*P. marginatum*), 8267 (*P. callosum*) and 8268 (*V. agnus-castus*).

**Extraction of essential oil**

Fresh leaves (500 g) of *P. marginatum* and *V. agnus-castus* and aerial parts (leaves, branches and inflorescences) of *P. callosum* (500 g) were subject to hydrodistillation for a period of 6 h in a Clevenger-type apparatus. The essential oils were centrifuged for 10 minutes at 3500 rpm to separate the water. After centrifugation, the water was removed with a pipette. The samples were stored in an amber bottle and kept under refrigeration until GC-MS analysis and insecticidal assays. The yields of the extractions were calculated on the basis of the oil volume and the weight of the plant material used.

**GC-MS analysis**

One microlitre of the extracted oils, dissolved in hexane (5.0 µL/mL), was injected and analysed by gas chromatography/mass spectrometry (GC-MS) on a Shimadzu QP-2010. The analyses were performed using a DB-5MS column (30 m x 0.25 mm, with an internal film thickness of 0.25 µm). The analysis was performed in electron impact ionization mode. The injector and interface were set at a temperature of 250°C while the oven was programmed with a temperature range from 60°C to 240°C (3°C/min). Helium (99.999% purity) was used as the carrier gas at a flow rate of 1.3 ml/min. The identification of the constituents was based on the interpretation of mass spectra by comparison with the library database Wiley 7, NIST 08 and FFNSC1.2, calculation of the linear retention index and comparison with the literature (Adams 2007). The linear retention indices were calculated using a homologous series of n-alkanes (Van Den Dool & Kratz 1963). The structures were computer-matched with the spectral libraries, and their fragmentation patterns were compared with literature data.

The relative concentrations of the chemical components of essential oils were obtained by normalization of the peak areas (%).

**Fumigant toxicity**

The evaluation of insecticide activity was performed at FCA/UNESP-Botucatu under the supervision of Prof. Dr. Edson Luiz Lopes Baldin. The essential oils (PM-EO, PC-EO and VA-EO) were applied to a filter paper attached to the bottom of the screw cap of a 50-mL glass vial (fumigation chamber) at concentrations of 0, 0.002, 0.004, 0.006, 0.008 and 0.01 µL/L air. Four replications were performed for each treatment. Five adult one-day-old *Z. subfasciatus* couples and 10 g of beans were transferred to the vials. The percent adult mortality was recorded 24, 48, and 72 h after treatment.

**Statistical analysis**

The results were subjected to analysis of variance by the F Test, and the means were compared by the Tukey test (*P*≤0.05) using the statistical software SISVAR version 5.6. The LD$_{50}$ was determined by Probit analysis using Stat Plus 2007 Professional Build 4.7.5.0. The Abbott formula was used to calculate the control efficiencies (Abbott 1987).

**RESULTS**

**Chemical constituents of the essential oil of PM-EO, PC-EO and VA-EO**

The essential oils of *Piper marginatum* (PM-EO), *Piper callosum* (PC-EO) and *Vitex agnus-castus* (VA-EO) extracted by hydrodistillation in Clevenger apparatus provided yields of 0.64%, 0.23% and 0.09% (mL/g), respectively.

The essential oils were obtained and analyzed by GC-MS to determine its composition. In the PM-EO sample, twenty-five compounds were identified, representing
81.8% of the total essential oil obtained, with predominance of hydrocarbon sesquiterpenes, followed by phenylpropanoids, hydrocarbon monoterpenes and oxygenated sesquiterpenes. The major compounds of PM-EO were 3,4-methylenedioxypropio Phenophenone (10.4%), bicyclogermacrene (10.1%) and germacrene D (9.9%) (Table I).

For PC-EO, twenty-four compounds representing 86.8% of the total essential oil obtained were identified, the majority being represented by hydrocarbon monoterpenes, followed by phenylpropanoids, hydrocarbon sesquiterpenes, oxygenated monoterpenes and oxygenated sesquiterpenes. Safrole (29.3%) α-pinene (19.2%) and β-pinene (14.3%) were the major compounds (Table I).

In the VA-EO sample, twenty-six substances were identified, representing 98.1% of the total essential oil obtained, the majority being oxygenated monoterpenes, followed by hydrocarbon sesquiterpenes, hydrocarbon monoterpenes and oxygenated sesquiterpenes. The major constituents were 1,8-cineol (23.8%), (E)-β-farnesene (14.6%), (E)-caryophyllene (12.5%) and sabinene (11.4%) (Table I).

**Fumigant toxicity of PM-EO, PC-EO and VA-EO**

The VA-EO sample killed 100% of the insects at a concentration of 0.004 µL/L air after 24 h of treatment (Table II). However, PC-EO killed 100% of insects after 48 h of treatment at a concentration of 0.01 µL/L air. The PM-EO sample was the least toxic against Z. subfasciatus. At a concentration of 0.01 µL/L air, PM-EO killed 55% of the insects after 24 h of treatment and caused 97.5% mortality after 72 h. Essential oils of the three species showed toxicity against Z.

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**Table I. Chemical constituents of the essential oil of P. marginatum, P. callosum and V. agnus-castus.**

| Compounds          | RIa | RIb | PM-EO (%) | PC-EO (%) | VA-EO (%) |
|--------------------|-----|-----|-----------|-----------|-----------|
| α-Thujene          | 924 | 924 | -         | 0.2       | 0.2       |
| α-Pinene           | 932 | 932 | 0.8       | 19.2      | 5.9       |
| Camphene           | 948 | 946 | -         | 0.6       | -         |
| Sabinene           | 971 | 969 | -         | 2.6       | 11.4      |
| β-Pinene           | 978 | 974 | 0.5       | 14.3      | 0.9       |
| Myrcene            | 988 | 988 | 0.5       | -         | 2.1       |
| α-Phellandrene     | 1007| 1002| -         | 0.1       | 0.6       |
| δ-3-Carene         | 1009| 1008| 4.2       | -         | -         |
| α-Terpinene        | 1016| 1014| -         | 1.4       | 0.3       |
| p-Cymene           | 1014| 1020| -         | 0.2       | 0.2       |
| Limonene           | 1028| 1024| -         | 0.8       | 2.8       |
| 1,8-Cineol         | 1031| 1026| -         | 2.2       | 23.8      |
| (Z)-β-Ocimene      | 1034| 1032| 3.8       | -         | -         |
| (E)-β-Ocimene      | 1044| 1044| 7.1       | -         | 0.6       |
| γ-Terpinene        | 1056| 1059| -         | 3.5       | 0.7       |
| Terpinolene        | 1084| 1088| -         | 0.8       | 0.3       |
| Terpinen-4-ol      | 1180| 1174| -         | 0.8       | 0.2       |
| α-Terpineol        | 1186| 1189| -         | 0.2       | 2.1       |
| Safrole            | 1290| 1287| -         | 29.3      | -         |
| Compound                                      | Retention Indices | Activity | Compound                                      | Retention Indices | Activity |
|-----------------------------------------------|-------------------|----------|-----------------------------------------------|-------------------|----------|
| δ-Elemene                                     | 1332, 1335        | 1.9      | α-Terpinyl acetate                            | 1346, 1352        | -        |
| Citronellyl acetate                           | 1350, 1354        | -        | α-Copaene                                     | 1372, 1374        | -        |
| β-Elemene                                     | 1386, 1389        | 0.6      | Methyl-eugenol                                | 1401, 1403        | 0.6      |
| α-Gurjunene                                   | 1409, 1409        | -        | β-Elemene                                     | 1386, 1389        | -        |
| (E)-Caryophyllene                             | 1415, 1417        | 5.0      | α-Humulene                                    | 1450, 1452        | 0.6      |
| (E)-β-Farnesene                               | 1454, 1456        | -        | Alloaromadendrene                             | 1458, 1461        | -        |
| Germacrene D                                  | 1476, 1480        | 9.9      | Bicyclogermacrene                             | 1490, 1500        | 10.1     |
| β-Selinene                                    | 1484, 1489        | 2.2      | (E)-Methyl-isoeugenol                         | 1496, 1491        | 0.5      |
| α-Muurolene                                   | 1495, 1500        | 1.2      | (E, E)-α-Farnesene                            | 1505, 1505        | -        |
| Germacrene A                                  | 1508, 1508        | 0.6      | Myristicin                                    | 1516, 1517        | 4.9      |
| δ-Cadinene                                    | 1518, 1523        | -        | Caryophyllene oxide                           | 1582, 1581        | -        |
| 3,4-Methylenedioxypropiophenone               | 1533, 1545        | 10.4     | Globulol                                      | 1590, 1590        | 0.5      |
| Elemicin                                      | 1548, 1555        | 8.4      | Viridiflorol                                  | 1592, 1590        | -        |
| Spathulenol                                   | 1575, 1577        | 1.5      | 10-epi-Eudesmol                               | 1622, 1622        | 0.6      |
| Caryophyllene oxide                           | 1582, 1581        | -        | Torreyol                                      | 1639, 1644        | 0.5      |
| β-Eudesmol                                    | 1650, 1650        | 4.1      | α-Cadinol                                     | 1652, 1653        | -        |
| α-Cadinol                                     | 1652, 1653        | -        | 3,4-Methylenedioxypropiophenone               | 1533, 1545        | 10.4     |
| Compound class                                |                   |          | 3,4-Methylenedioxypropiophenone               | 1533, 1545        | 10.4     |
| Monoterpene hydrocarbons                      | 16.9, 42.6        | 26.0     | Oxygenated monoterpenes                       | 0.0, 2.9          | 36.4     |
| Oxygenated monoterpenes                       | 0.0, 2.9          | 36.4     | Total monoterpenes                            | 16.9, 45.5        | 62.4     |
| Sesquiterpene hydrocarbons                    | 32.5, 7.0         | 34.5     | Oxygenated sesquiterpenes                     | 7.4, 2.0          | 1.2      |
| Sesquiterpene hydrocarbons                    | 32.5, 7.0         | 34.5     | Total sesquiterpenes                          | 39.9, 9.0         | 35.7     |
| Phenylpropanoids                              | 25.0, 32.3        | 0.0      | Total identified                              | 81.8, 86.8        | 98.1     |

Ri= Retention Indices relative to n-alkanes (C8-C25) on the DB-5MS column.
Ri= Retention Indices of the literature (Adams 2007).
subfasciatus. After 24 h of treatment, the VA-EO sample had the lowest LD₅₀ (0.17 µL/L air), followed by PC-EO with an LD₅₀ of 0.78 and PM-EO with an LD₅₀ of 1.17 µL/L air (Table III).

DISCUSSION

The chemical composition of PM-EO was different from other specimens reported in the literature. For the specimen occurring in the Brazilian Amazon, (E)-β-ocimene (13.5 %), myristicin (9.3 %), β-caryophyllene (6.0 %), (Z)-β-ocimene (5.3 %) and 3,4-methylenedioxyphenophenone (5.0 %) were reported as major constituents (Andrade et al. 2008). The essential oil collected in the State of Pará presented myristicin (15.75%) and α-cadinene (7.98%) as major constituents (Krinski et al. 2018). However, the main compounds in the essential oil of a specimen collected in State of Paraíba, Brazil were isoelemicin (21.7%) and apiole (20.1%) (Costa et al. 2010). The essential oil of leaves of *Piper marginatum*, harvested in the Atlantic forest in the State of Pernambuco, Brazil, presented (Z)-asarone (30.4%) and patchouli alcohol (16.0%) as major constituents (Autran et al. 2009). In the present study, we have detected 3,4-methylenedioxypropiophenone (10.4%), bicyclogermacrene (10.1%) and germacrene D (9.9%) as the major constituents of PM-EO.

For PC-EO, the major constituents were safrole (29.3%), α-pinene (19.2%) and β-pinene

| EO tested doses (µL/L air) 24 h | Exposure time |
|-------------------------------|---------------|
|                               | 48 h          | 72 h          |
| PM-EO                         |               |               |
| 0.002                         | 25.0±0.5 b    | 10.0±0.2 b    | 22.5±1.5 b |
| 0.004                         | 0.0±0.0 b     | 0.0±1.0 b     | 17.5±2.1 b |
| 0.006                         | 0.0±0.0 b     | 50.0±3.2 a    | 77.5±2.6 a |
| 0.008                         | 50.0±1.0 b    | 40.0±4.7 a    | 60.0±0.8 a |
| 0.01                          | 55.0±5.2 a    | 60.0±6.7 a    | 97.5±0.5 a |
| PC-EO                         |               |               |
| 0.002                         | 0.0±0.0 d     | 7.5±0.9 cd    | 17.5±0.9 c |
| 0.004                         | 10.0±0.8 cd   | 32.5±2.2 bc   | 50.0±1.6 b |
| 0.006                         | 30.0±1.15 bc  | 67.5±2.1 ab   | 80.0±1.4 a |
| 0.008                         | 42.5±2.63 bc  | 77.5±2.2 ab   | 100.0±0.0 a|
| 0.01                          | 85.0±1.00 a   | 100.0±0.0 a   | 100.0±0.0 a|
| VA-EO                         |               |               |
| 0.002                         | 65.0±3.7 a    | 87.5±1.5 a    | 92.5±0.9 a |
| 0.004                         | 100.0±0.0 a   | 100.0±0.0 a   | 100.0±0.0 a|
| 0.006                         | 95.0±0.6 a    | 95.0±0.6 a    | 100.0±0.0 a|
| 0.008                         | 97.5±0.5 a    | 100.0±0.0 a   | 100.0±0.0 a|
| 0.01                          | 100.0±0.0 a   | 100.0±0.0 a   | 100.0±0.0 a|

For each exposure period, the percentages with the same letters do not differ by the Tukey test (P > 0.05).
(14.3%) (Table I). The chemical composition of the PC-EO sample was similar to those of other specimens from other locations (Maia et al. 1987, Almeida et al. 2018), but the concentrations of the compounds were different. For example, the essential oil obtained from leaves collected on the AM-010 road Manaus-Itacoatiara presented safrole (64.0%), β-pinene (12.9%) and α-pinene (6.9%) as major compounds (Maia et al. 1987). Safrole (59.1%), β-pinene (8.3%) and α-pinene (6.5%) were also the major compounds found in the essential oil of a specimen collected in Manaus (Almeida et al. 2018). However, the major compounds of PC-EO were different from those of the specimen collected in Peru, with asaricin (35.9%), safrole (20.2%), methyl-eugenol (9.7%) and (E)-asarone (7.8%) reported as main constituents (Van Genderen et al. 1999).

The diversity in the chemical composition of the essential oils collected in different regions can be attributed to several biotic and abiotic factors (Gobbo-Neto & Lopes 2007).

The major constituents of VA-EO were 1,8-cineol (23.8%), (E)-β-farnesene (14.6%), (E)-caryophyllene (12.5%) and sabinene (11.4%) (Table I). The chemical composition of VA-EO was similar to those of other specimens reported in the literature (Neves & Camara 2016, Zoghbi et al. 1999). 1,8-cineol (17.6%) and (E)-β-farnesene (13.6%) were the major constituents of essential oil obtained in the State of Pernambuco, Brazil (Neves & Camara 2016). In the essential oil of a specimen collected in the Amazon, the major compounds were 1,8-cineol (33.5%) and sabinene (18.5%) (Zoghbi et al. 1999).

The results obtained in the toxicity test against *Z. subfasciatus* were similar to those obtained with the essential oil of *Chenopodium ambrosioides* L., which killed 100% *Z. subfasciatus* after 12 h of treatment at a concentration of 20.0 µL/L air (LD₅₀ of 0.8 µL/L air after 24 h exposure) (Bernardes et al. 2018). Several studies have demonstrated the fumigant action of essential oils against *Callosobruchus maculatus* (Coleoptera: Bruchidae) (Mahmoudvand et al. 2011), *Sitophilus granarius* (L.) (Hamza et al. 2016), *Callosobruchus maculatus* F., *Sitophilus zemais* Motchulsky and *Rhyzopertha dominica* F. (Gragasin et al. 2006), which are all insects that attack stored grain products. The fumigant

| EO      | Exposure time | LD₅₀/CI     | Slope±SE | X²    |
|---------|---------------|-------------|----------|-------|
| PM-EO   | 24 h          | 1.17 (0.9-1.3) | 4.1 ± 0.7 | 38.0  |
|         | 48 h          | 0.82 (0.7-0.8) | 3.4 ± 0.5 | 14.6  |
|         | 72 h          | 0.56 (0.4-0.6) | 3.5 ± 0.5 | 2.4   |
| PC-EO   | 24 h          | 0.78 (0.7-0.8) | 6.4 ± 1.5 | 14.8  |
|         | 48 h          | 0.53 (0.4-0.5) | 2.8 ± 0.5 | 1.7   |
|         | 72 h          | 0.41 (0.3-0.4) | 2.3 ± 0.3 | 0.6   |
| VA-EO   | 24 h          | 0.17 (0.04-0.2) | 2.8 ± 0.6 | 4.1   |
|         | 48 h          | *            | *        | *     |
|         | 72 h          | 0.04 (-0.09-0.1) | 2.1 ± 0.8 | 5.9   |

LD, Lethal dose (µL/L air); CI, Confidence interval at 95% probability; X², Chi square test. * Data are absent because the insects died within the first hour of the experiment.
action of VA-EO, PC-EO and PM-EO is of great importance as essential oils are relatively safer and greener. In addition, botanical insecticides are more relatively economically viable to conserve stored grains and manage pests in accordance with international biosafety regulations (Bernardes et al. 2018).

The fumigant action of essential oils may be closely linked to their chemical composition, since monoterpenoids are reported as inhibitors of the enzyme acetylcholinesterase (AChE), (Houghton et al. 2006, Rajendran & Sriranjini 2008) responsible for the breakdown of acetylcholine, a neurotransmitter of nerve impulses. (Bruneau & Akaaboune 2006). Studies following the reports of AChE inhibition by the oils of two species of sage, Salvia officinalis and S. lavandulaefolia (Lamiaceae) led to the discovery that 1,8-cineol and α-pinenene were the most active compounds with IC₅₀ 670 µM and 630 µM respectively against bovine erythrocyte AChE (Houghton et al. 2006). Besides that, trans-cinnamaldehyde, eugenol, (-)-menthane and (-)-terpinen-4-ol monoterpenes were evaluated and showed a reduction in the progeny of Sitophilus oryzae after 45 and 90 days of treatment (Saad & Abdelgaleil 2018).

Some of the purified terpenoid constituents of essential oils are moderately toxic to mammals, but, with few exceptions, the oils themselves or products based on oils are mostly nontoxic to mammals, birds, and fish. In addition, due to their volatility, essential oils have limited persistence in field conditions (Isman 2006). In turn, PM-EO and PC-EO contain the compounds 3,4-methylenedioxypropiophenone and safrole, respectively. These substances have the methylenedioxyphenyl group, characteristic of many other compounds derived from secondary metabolism of plants, as dillapiole occurring in P. aduncum, as well as piperine and piperolein existing in P. nigrum, traditionally used as botanical insecticides (Mukerjee et al. 1979, Scott et al. 2008). In addition, the phenylpropanoid safrole, major constituent of PC-EO, was responsible for the fumigant action on Sitophilus zeamais Motschulsky and Tribolium castaneum, stored grain pests (Huang et al. 1999). In addition, the complex mixture of chemical substances present in the studied essential oils can be directly related to the insecticidal activity, as they can act synergistically. Therefore, interest in plant products such as essential oils and their compounds has increased in recent years due to their fumigant action, as it is believed that natural compounds from plant sources may have advantages over conventional fumigants in terms of low toxicity in mammals, rapid degradation and regional availability. (Rajendran & Sriranjini 2008).

**CONCLUSIONS**

Essential oils of Piper marginatum, Piper callosum and Vitex agnus-castus showed fumigant toxicity on Z. subfasciatus, with VA-EO being the most toxic, followed by PC-EO and PM-EO. There was no oviposition in the treatments and exposure times evaluated. The results suggest that essential oils can be used as an environmentally friendly alternative to control pests in stored products. However, further studies are needed to determine which compounds may be responsible for such activities.

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Vanessa F. dos S. Ayres, Geone M. Correa, Anderson C. Guimarães and Renata Takeara contributed collecting plant sample and essential oil extraction. Vanessa F. dos S. Ayres and Midiã R. de Oliveira contributed in the chemical analysis of the essential oils. Vanessa F. dos S. Ayres, Midiã R. de Oliveira and Renata Takeara contributed in the insecticidal assay and to analysis of the data.