An Acad Bras Cienc (2022) 94(2): e20200072 DOI 10.1590/0001-3765202220200072
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CROP SCIENCE

Lethal and sublethal effects of essential oils from *Piper capitarianum* Yunck and *Piper krukoffii* Yunck on *Plutella xylostella* L.

MILENA L.G. SANTANA, JOÃO P.R. DE MELO, CLÁUDIO A.G. DA CAMARA, MARCÍLIO M. DE MORAES, CAROLINA A. DE ARAUJO, GERALDO J.N. DE VASCONCELOS, MARTA R.S. PEREIRA & CHARLES E. ZARTMAN

Abstract: *Plutella xylostella* (L.) is responsible for considerable vegetable crop losses in the metropolitan region of Manaus, Brazil. In recent decades, essential oils have been investigated as an alternative to synthetic insecticides. The genus *Piper* is widely distributed in Amazonia and essential oils from these plants have insecticidal properties. This study describes the chemical composition of the essential oils from *Piper capiterianum* and *Piper krukoffii* as well as the lethal and sublethal effects on *P. xylostella*. The phytotoxicity of the oils on the host plant was also evaluated. Globulol was the major constituent of the *P. krukoffii* oil and *o*-cymene was the major constituent of the *P. capitarianum* oil. The oil from *P. capiterianum* exhibited greater toxicity to larvae and eggs. This oil also presented greater repellant action, feeding deterrence and mild phytotoxicity to the host plant (*Brassicae oleraceae*). The findings suggest that this oil can be used in the preparation of a formulated insecticide for the management of *P. xylostella* in different development phases. However, further studies are needed to evaluate the effect of this oil on crops under field conditions as well as non-target organisms and determine the cost-benefit ratio of a product formulated with *P. capitarianum* oil.

Key words: *Plutella xylostella*, larvicide, ovicide, repellency, feeding deterrence, phytotoxicity.

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the major pest of *Brassica* (cruciferous) crops throughout the world (Zalucki et al. 2012). This cosmopolitan pest is distributed from the cold mountains of Himalaya to hot, dry regions of Ethiopia (Mohan et al. 2009). It also occurs throughout the entire country of Brazil (Castelo Branco & França 2015). In 2012, the total annual cost related to the management of *P. xylostella* for the protection of cruciferous crops was US$ 4 to 5 billion and this figure was US$ 17 million in Brazil alone (Zalucki et al. 2012).

According to the Brazilian Seed and Seedling Association, the Brazilian production of cabbage surpassed 1.4 million tons in 2017/2018. However, crop losses are quite high in some locations of the northern region of the country due to attacks from agricultural pests, especially *P. xylostella* in the São Francisco/Terra Nova farming community, which is located in the metropolitan region of Manaus in the state of Amazonas, Brazil. The main form of pest control in this community consists of the application of synthetic insecticides,

such as chloranthraniliprole, cyantraniliprole, chlorfenapyr and deltamethrin, the latter of which is less costly for small farmers. However, the indiscriminate use of these products has caused serious harm to non-target organisms, such as natural enemies, and has led to the emergence of resistant pest populations (Moraes & Marinho-Prado 2016). The insecticides used in Brazil include chlorantraniliprole, cyantraniliprole, chlorfenapyr and deltamethrin, for which there are reports of resistant populations of *P. xylostella* (Ribeiro et al. 2017, Lima Neto et al. 2016, Oliveira et al. 2011). In recent decades, the use of plant-based insecticides has become an ecologically viable alternative to synthetic products. Such products can be obtained from the leaves, flowers and stems of plants and used in the form of powders, extracts and essential oils, the effectiveness of which has been proved in several studies (de Melo & da Camara 2019, Bandeira et al. 2013, Silva et al. 2018).

Brazil is the country with the greatest vegetal genetic diversity and is home to 30% of all the tropical forests on the planet (Lewinsohn & Prado 2005). According to Maia & Andrade (2009), among the 280 medicinal plant species cataloged from the Amazon, approximately 40% belong to the family Piperaceae. The genus *Piper* is one of the largest in the family, with 2000 species encountered in tropical and temperate regions in both hemispheres (Quijano-Abril et al. 2006). Among the 290 species of *Piper* found in Brazil, 137 have been recorded for the state of Amazonas (AM) (Guimarães et al. 2015). Plants of this genus stand out for their production of essential oils, amides and phenylpropanoids, which have insecticidal properties that affect hemipterans (Piton et al. 2014), lepidopterans (Lima et al. 2009), dipterans (Santana et al. 2015) and coleopterans (Pereira et al. 2008). Among the species that occur in the Amazon, *Piper krukoffii* Yunck and *Piper capitarianum* Yunck stand out by its broad distribution and biological properties, such as antioxidant activity and larvicidal action against the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae) (da Silva et al. 2011, França 2015). However, no previous study has evaluated the lethal and sublethal effects of essential oils from the leaves of these species on *P. xylostella*, which is an important agricultural pest that affects cruciferous crops in the community of São Francisco/Terra Nova in metropolitan Manaus, Brazil.

Giving continuity to the chemical and biological study of essential oils from aromatic species with insecticidal potential, the aim of the present study was to determine the chemical composition of the essential oils from the leaves of *P. krukoffii* and *P. capitarianum* collected from the Amazon biome and evaluate the effects on *P. xylostella* in terms of mortality (eggs and larvae), feeding deterrence and repellent action to enable the formulation of a plant-based insecticide containing these *Piper* oils as the main ingredient. The phytotoxicity of the oils to the host plant was also investigated. The results were compared to those obtained with commercial plant-based (Azadirachtin) and synthetic (Deltamethrin) insecticides used as positive controls.

MATERIALS AND METHODS

Collection of plant material

Leaves from *Piper krukoffii* and *Piper capitarianum* were collected from the ISB/UFAM reserve in Coari, AM, Brazil (04°07'20"S 63°04'29"W) and along roadway BR-174 in Manaus, AM, Brazil (02°50'24"S 60°01'58"W), respectively. The plants were identified by botanist M.R. Pereira (National Institute for Amazonian Research). Vouchers of both samples were mounted and deposited in the herbarium of the *Instituto Nacional de Pesquisas da Amazônia*

(INPA) under numbers 685 (*P. capitarianum*) and 700 (*P. krukoffii*).

Isolation of essential oils

Essential oils from the leaves of *P. krukoffii* (100 g) and *P. capitarianum* (100 g) were obtained by hydrodistillation using a modified Clevenger apparatus for 4 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers and kept at a low temperature (-5 °C) until analysis and the assays. Total oil yields were expressed as percentages (g/100 g of fresh plant material). All experiments were carried out in triplicate.

Chemicals

The chemicals used as standards for the identification of volatile compounds in the oils were purchased from Sigma-Aldrich (Brazil). Deltamethrin (Decis® 25 g i.a./L EC Bayer CropScience) and azadirachtin (Azamax® 12 g i.a./L EC E.I.D. Parry) were acquired from the local market and used as positive controls.

Gas chromatography fid analysis

Gas chromatography (GC) was performed using a Hewlett-Packard 5890 Series II GC apparatus equipped with a flame ionization detector (FID) and a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness) (J and W Scientific). The oven temperature was programmed from 60 to 240 °C at a rate 3 °C min^{-1}. Injector and detector temperatures were 260 °C. Hydrogen was used as the carrier gas at a flow rate of 1 ml min^{-1} in split mode (1:30). The injection volume was 0.5 µL of diluted solution (1/100) of oil in n-hexane. The percentage of each compound was obtained from GC-FID peak areas in the order of the DB-5 column elution and expressed as the relative percentage of the area of the chromatograms. The analysis were performed in triplicate.

Gas chromatography-mass spectrometry (gc-ms) analysis

The GC-MS analysis of the essential oils was carried out using a Varian 220-MS IT GC system with a mass selective detector mass spectrometer in EI 70 eV with a scan interval of 0.5 s and fragments from 40 to 550 Da. fitted with the same column and temperature program as used for GC-FID with the following parameters: carrier gas = helium; flow rate = 1 ml min^{-1}; split mode (1:30); injected volume = 1 µL of diluted solution (1/100) of oil in n-hexane.

Identification of components

Identification of the components was based on GC-MS retention indices with reference to a homologous series of C_8-C_{40} n-alkanes calculated using the van Den Dool and Kratz equation (van Den Dool & Kratz 1963) and by computer matching against the mass spectral library of the GC-MS data system (NIST version 14 and WILEY version 11), co-injection with authentic standards and other published mass spectra (Adams 2007). Area percentages were obtained from the GC-FID response without the use of an internal standard or correction factors.

Acquisition and rearing of *Plutella xylostella*

Specimens of *P. xylostella* were originally collected from collard greens (*Brassica oleracea* var. *acephala*) in 2015 in the municipality of Recife, state of Pernambuco, Brazil (08º 01'08.3" S 34º 56' 45.5" W) and maintained at the Laboratory for the Chemical Investigation of Natural Insecticides of UFRPE, Brazil, with approximately 60 generations having occurred by 2019. The moths were reared at a temperature of 25 ± 1 ºC, relative humidity of 65 ± 5% and a 12-h photoperiod and without any exposure to insecticides. The breeding method was adapted from Bandeira et al. (2013).

Larvicidal assay

The residual effect bioassays were based on the method described by Bandeira et al. (2013). Experiments were performed with open Petri dishes (10 cm diameter). Leaf discs (2.5 cm diameter) cut from collard greens were immersed for 30 seconds in the solutions prepared with essential oil, diluted in the solvent to dissolve (WPDA = distilled Water + 1.0% Polyoxyethylene sorbitan monolaurate + 0.1% Dodecylbenzenesulfonic Acid), using the immersion method and allowed to dry on a paper towel at room temperature for 30 minutes. Ten third instar *P. xylostella* larvae were placed on each dish. The experimental design was entirely randomized, totaling 120 larvae per treatment. The concentrations ranged from 0.0035 to 1.90 mg ml^{-1} (*P. capitarianum*), 1.02 to 24.50 mg mL^{-1} (*P. krukoffii*), 0.003 to 0.200 mg mL^{-1} (Deltamethrin) and 0.003 to 0.300 mg mL^{-1} (Azadirachtin). Mortality was recorded after 48 hours of exposure. Specimens with no sign of movement were considered dead. Negative control disks were only immersed in the WPDA solvent.

Ovicidal assay

The ovicidal bioassay was the same as that employed by Zago et al. (2010). Ten newly emerged adult males and females in pairs were placed in screened recipients containing leaf disks (2.5 cm diameter) of collard greens for oviposition. At six-hour intervals, the leaf disks were removed from the recipients. Thirty eggs were counted and the remaining eggs were removed. Leaf disks with 30 eggs were immersed for 30 seconds in different concentrations of the essential oils and positive controls (Azamax® and Deltamethrin) diluted in WPDA solvent. The concentrations ranged from 0.01 to 0.25 mg ml^{-1} (*P. capitarianum*), 0.5 to 6.0 mg mL^{-1} (*P. krukoffii*), 0.005 to 0.25 mg mL^{-1} (Azadirachtin) and 0.003 to 1.5 mg mL^{-1} (Deltamethrin). Negative control disks were only immersed in the WPDA solvent. After drying at room temperature for 30 minutes, the leaf disks with the eggs were placed on filter paper on sponge saturated with water in plastic trays and kept in a climatic chamber (BOD MA 403) at 25 ± 1°C and 70 ± 10% relative humidity. Egg viability was evaluated 96 hours after exposure to the substances by counting the number of hatched larvae.

Antifeedant bioassays

The feeding deterrence method was adapted from Akhtar et al. (2012). Third instar *P. xylostella* larvae were transferred to Petri dishes and deprived of food for four hours prior to the experiments. Collard leaf disks (2.0 cm diameter) were immersed for 30 seconds in the solutions prepared with essential oil and positive control, diluted in WPDA solvent and allowed to dry on a paper towel at room temperature. Control disks were only immersed in distilled water. The concentrations ranged from 0.01 to 0.35 mg ml^{-1} (*P. capitarianum*), 1.2 to 9.59 mg mL^{-1} (*P. krukoffii*) and 0.01 to 0.97 mg mL^{-1} (Azadirachtin). After drying, a treated disk and control disk were placed at a distance of 2.0 cm in each Petri dish. A larva was placed in the center of the Petri dish between the two leaf disks and allowed to feed for 24 h. Thirty repetitions were used for each treatment, with each repetition consisting of one larvae. After 24 h of exposure, the larvae were removed. The areas of the leaves consumed in the control and treatment disks were determined with the aid of the Licor-3100 leaf area meter, which has high accuracy and repeatability, with reading resolution ranging from 0.1 to 1 mm^2. The feeding deterrence index (FDI) was calculated as follows: $FDI = 100\{(C - T) / (C + T)\}$, in which C and T are the areas consumed on the control and treated disks, respectively.

The results were compared to those obtained with the positive control (Azadirachtin).

Larval repellency bioassay

The larval repellency bioassay was adapted from Lobo et al. (2019). Collard leaf disks (2.0 cm diameter) were immersed for 30 seconds in the solutions prepared with sublethal concentrations of the essential oils and positive control diluted in WPDA solvent. Control disks were only immersed in distilled water. The material was set to dry on paper towels at room temperature for 30 minutes. After drying, a treated disk and control disk were placed at a distance of 2.0 cm in each Petri dish. A third instar *P. xylostella* larvae was placed in the center of the Petri dish between the two leaf disks. Thirty repetitions were used for each treatment, with each repetition consisting of one Petri dish containing one larva. The repellent effect was evaluated 1, 2, 4, 6, 12 and 24 hours after the onset of the experiment, with the recording of the number of *P. xylostella* larvae on the treatment and control disk leaves. The sublethal concentrations for the evaluation of the repellency index were 0.02, 0.04, 0.06 and 0.07 mg ml^{-1} (*P. capitarianum*), 1.20, 1.74, 2.23 and 2.72 mg ml^{-1} (*P. krukoffii*) and 0.007, 0.010, 0.015 and 0.020 mg ml^{-1} (Azadirachtin).

The repellency index (RI) was calculated as follows: RI = 2G / (G + P), in which G is the % of larvae found on the leaf disks treated with the essential oil or positive control and P is the % of larvae on the control leaf disks. The RI ranges from 0 to 2. RI = 1 denotes neutral action, RI > 1 denotes attraction and RI < 1 denotes repellency. As the margin of safety for this classification, the standard error (SE) of each treatment was added to or subtracted from 1.00 (index indicative of neutrality). Thus, each treatment was only considered repellent or attractive when the RI was outside the 1.00 ± SE range (Mazzonetto & Vendramim 2003). A repellency intensity scale based on Bustos et al. (2017) was used for the classification of the degree of repellency of the essential oils and positive control to *P. xylostella* larvae (0.76-0.99 = weak; 0.51-0.75 = moderate; 0.26-0.50 strong; 0.00-0.25 very strong).

Phytotoxicity test

The method for the phytotoxicity test was adapted from Torres et al. (2006). Collard leaf disks (5 cm diameter) were immersed for 10 s in the essential oils diluted in WPDA solvent and set to dry at room temperature. After 48 h, the phytotoxicity index (PI) of each leaf disk was evaluated with the aid of the AFSoft program. The images were analyzed using criteria of the phytotoxicity scale proposed by Alvez et al. (1974): 0.00 to 4.90% = slight; 5.00 to 14.99% = mild; 15.00 to 29.99% = acceptable; 30.00 to 39.99% = borderline acceptable; 40.00 to 100.00% = severe. The PI was calculated using the following formula: PI = TA% - SA%, in which TA is total area and SA is the area of sound (unaffected) leaf. The phytotoxic assessment was performed with the greatest concentration of essential oil and positive control (Azadirachtin and Deltamethrin) used in the toxicity bioassays.

Statistical analysis

To estimate the curve slopes, the results of the larvicidal and ovicidal assays, LC_{50} (lethal concentration) and FDI_{50} (lethal concentration) of each *Piper* oil and positive control were submitted to PROBIT analysis (Finney 1971) using SAS software (version 9.0) (SAS 2002). The concentrations were calculated based on the logarithmic series proposed by Robertson et al. (2017). The data from the repellency bioassays were submitted to analysis t-test using PROC TTEST SAS, with the means compared by the X^2 estimated using the Statistical Analysis System software (SAS 2002).

RESULTS

Chemical analysis and identification of constituents of essential oils

The yields and percentages of the chemical constituents identified in the *Piper* oils are displayed in Table I. Hydrodistillation of the leaves of the two species analyzed provided yellowish oils with a citric aroma. The yields were 0.49 ± 0.05% for *P. krukoffii* and 0.23 ± 0.02% for *P. capitarianum*.

The GC-MS analysis enabled the identification of 28 and 27 compounds in the oils of *P. krukoffii* and *P. capitarianum,* respectively representing 98.42 ± 0.83% and 96.60 ± 0.75% of the total oil. Among the compounds identified in the oils, only dehydro-aromadendrene [*P. krukoffii* (2.45 ± 0.05%) and *P. capitarianum* (12.32 ± 0.29%)] and pogostol [*P. krukoffii* (3.51 ± 0.08%) and *P. capitarianum* (4.00 ± 0.10%)] were found in both oils (Supplementary Material - Figure S1). These data suggest qualitative and quantitative differences in the chemical composition of the two oils.

Globulol (17.54 ± 0.07%) followed by 4-epi-cis-dihydroagarofuran (12.25 ± 0.23%) and γ-muurolene (11.03 ± 0.21%) were the major constituents in the *P. krukoffii* oil, whereas o-cymene (40.74 ± 0.97%) followed by dehydro-aromadendrene (12.32 ± 0.29%) and β-chamigrene (9.96 ± 0.24%) were the major constituents of the *P. capitarianum* oil. The *P. krukoffii* oil was composed mainly of sesquiterpenes (97.25 ± 0.12%), while in *P. capitarianum* oil the content of monoterpenes and sesquiterpenes was very similar. No phenylpropanoids were identified in the *P. capitarianum* oil and methyl eugenol (1.17%) was the only compound from this chemical class found in the *P. krukoffii* oil in the present investigation.

P. Xylostella larvicidal and ovicidal bioassay

Table II displays the mean lethal concentrations (LC_{50}) of the essential oils from the leaves of *P. krukoffii* and *P. capitarianum* and the positive controls (Azadirachtin and Deltamethrin) for *P. xylostella* larvae and eggs. The susceptibility of the pest varied in accordance with the plant species from which the oil was extracted and the development phase of the pest. The *P. capitarianum* oil (LC_{50} for larvae = 0.21 mg mL^{-1} and eggs = 0.079 mg mL^{-1}) was respectively 30.3-fold and 33.9-fold more toxic to larvae and eggs than the *P. krukoffii*. Moreover, based on the LC_{50}, eggs were respectively 4.60-fold and 3.44-fold more susceptible to the *P. capitarianum* and *P. krukoffi* oils than third instar larvae. These results show that the essential oils from *P. capitarianum* and *P. krukoffi* are promising as active ingredients in the formulation of a natural insecticide for the control of the different developmental forms of *P. xylostella*. However, it is necessary to test of the constituents, found in the oils, separately or in the form of mixtures.

In the comparison of relative toxicity, both *Piper* oils were less toxic than the positive controls (Azadirachtin and Deltamethrin) to the *P. xylostella* larvae. Regarding ovicidal activity, only the *P. capitarianum* oil had the same effectiveness as Deltamethrin, whereas Azadirachtin had greater ovicidal action than both *Piper* oils.

Feeding deterrence and larval repellency bioassay

The *P. capitarianum* and *P. krukoffii* oils applied to the collard leaves were capable of reducing the feeding of the *P. xylostella* larvae at sublethal concentrations (Table III). The *P. capitarianum* oil exhibited greater deterrent action, reducing the feeding of the *P. xylostella* larvae 46-fold more than the *P. krukoffii* oil. Moreover, the *P. capitarianum* oil was approximately twofold more efficient than the plant-based commercial

Table I. Chemical composition of essential oils from leaves of *Piper krukoffi* and *Piper capitarianum*.

Compounds	RIL	RIC	*P. krukoffii* % ± S.E.	*P. capitarianum* % ± S.E.
Yield (%)±S.E.			0.49 ± 0.05	0.23 ± 0.02
Tricyclene[‼]	929	921	-	0.92 ± 0.02
β-Pinene[‡‡]	974	974	-	0.55 ± 0.32
6-methyl-5-Hepten-2-one[‼]	978	981	-	0.88 ± 0.02
Myrcene[‡‡]	985	988	-	0.73 ± 0.02
o-Cymene[‡‡]	1024	1022	-	40.74 ± 0.97
γ-terpinene[‡‡]	1054	1054	-	2.73 ± 0.06
m-Cymene[‼]	1086	1082	-	0.45 ± 0.26
Terpinen-4-ol[‼]	1176	1174	-	0.50 ± 0.29
p-Cymen-8-ol[‼]	1181	1179	-	0.87 ± 0.02
Linalool formate[‼]	1218	1214	-	0.58 ± 0.34
trans-Ascaridol glycol[‼]	1267	1266	-	0.86 ± 0.02
p-Cymen-7-ol[‼]	1286	1289	-	1.40 ± 0.04
trans-tetrahydro Jasmone[‼]	1296	1309	-	0.64 ± 0.02
δ-Terpinyl acetate[‼]	1309	1316	-	1.43 ± 0.03
(*E*)-Jasmonol[‼]	1315	1322	-	1.96 ± 0.05
neoiso-Carvomethyl acetate[‼]	1344	1347	-	0.61 ± 0.35
α-Ylangene[‼]	1366	1373	1.76 ± 0.04	-
α-Copaene[‼]	1370	1374	-	0.80 ± 0.02
β-Bourbonene[‼]	1383	1387	0.87 ± 0.02	-
β-Elemene[‼]	1385	1389	5.34 ± 0.10	-
methyl Eugenol[‼]	1400	1403	1.17 ± 0.02	-
Longifolene[‼]	1401	1407	0.55 ± 0.32	-
(*Z*)-Caryophyllene[‼]	1403	1408	6.75 ± 0.13	-
β-Duprezianene[‼]	1426	1421	-	1.78 ± 0.04
β-Gurjunene[‼]	1428	1431	0.57 ± 0.33	-
α-Guaiene[‼]	1431	1437	1.30 ± 0.03	-
α-Himachalene[‼]	1448	1449	-	1.82 ± 0.04
α-Humulene[‡‡]	1450	1452	1.56 ± 0.03	-
Allo-Aromadendrene[‼]	1455	1458	1.21 ± 0.03	-
dehydro-Aromadendrene[‼]	1458	1460	2.45 ± 0.05	12.32 ± 0.29
9-*epi*-(*E*)-Caryophyllene[‼]	1463	1464	1.00 ± 0.02	-
β-Chamigrene[‼]	1474	1476	-	9.96 ± 0.24
γ-Muurolene[‼]	1475	1478	11.03 ± 0.21	-
β-Selimene[‼]	1485	1489	2.60 ± 0.05	-
δ-Selimene[‼]	1491	1492	3.02 ± 0.06	-
epi-Cubebol[‼]	1492	1493	4.95 ± 0.10	-
γ-Amorphene[‼]	1498	1495	-	0.65 ± 0.02

Table I. Continuation.

4-*epi*-*cis*-Dihydroagarofuran[‼]	1498	1499	12.25 ± 0.23	-
α-Muurolene[‼]	1500	1500	0.54 ± 0.31	-
trans-*β*-Guaiene[‼]	1501	1502	1.47 ± 0.03	-
δ-Amorphene[‼]	1509	1511	-	0.96 ± 0.02
Hedycaryol[‼]	1547	1546	1.39 ± 0.03	-
(*E*)-Nerolidol[‡‡]	1560	1561	1.09 ± 0.03	-
Longipinanol[‼]	1566	1567	1.94 ± 0.34	-
Caryophyllene oxide[‡‡]	1580	1582	-	5.96 ± 0.14
Globulol[‡‡]	1589	1590	17.54 ± 0.07	-
Viridiflorol[‼]	1593	1592	-	0.87 ± 0.02
Longiborneol[‼]	1600	1599	-	0.88 ± 0.02
Guaiol[‼]	1601	1600	3.50 ± 0.09	-
β-Eudesmol[‼]	1650	1649	4.65 ± 0.07	-
Pogostol[‼]	1652	1651	3.51 ± 0.08	4.00 ± 0.10
epi-Zizanone[‼]	1665	1668	3.77 ± 0.34	-
Helifolenol A[‼]	1675	1674	0.60 ± 0.83	-
Total			98.42 ± 0.83	95.85 ± 0.75
Monoterpenes			-	55.85 ± 0.95
Sesquiterpenes			97.25 ± 0.82	40.00 ± 0.20
Phenylpropanoids			1.17 ± 0.02	-

SE= Standard Error; RIL =Retention indices from the literature; RIC=Retention indices calculated from retention times in relation to those of a series of C_8 –C_{40} n-alkanes on a DB-5 capillary column; [‼]Method of identification: Retention Index and Mass Spectroscopy. [‡‡]Method of identification: Retention Index; Mass Spectroscopy; Co-Injection with authentic compounds.

insecticide (Azadirachtin) used as the positive control. The results show that these essential oils are promising as a plant-based insecticide for use in the management of *P. xylostella* larvae.

Regarding the repellent action, the *P. capitarianum* oil exhibited high to moderate degrees of repellency at 0.02, 0.04, 0.06 and 0.07 mg mL^{-1} to 3rd instar larvae of *P. xylostella* in the first 12 hours after exposure to the oil. After 24 hours, the larvae began to be attracted to the *P. capitarianum* oil (RI > 1) (Figure 1). The *P. krukoffii* oil at 1.20, 1.74 and 2.23 mg mL^{-1} was repellent only in the first hour after application. At 2.72 mg mL^{-1}, however, this oil was repellent for 12 h (Figure 2). The positive control Azadirachtin was attractive to the larvae throughout the evaluation period at all concentrations tested (0.007, 0.010, 0.015 and 0.020 mg ml^{-1}) (Figure 3).

Phytotoxicity bioassay

Table IV displays the phytotoxicity of the essential oils to collard greens (*Brassica oleraceae* var. acephala). Based on the phytotoxicity scale proposed by Alvez et al. (1974), the *P. capitarianum* and *P. krukoffii* respectively exhibited mild (9.83%) and acceptable (16.68%) toxicity to the host plant. Considering the positive controls, the essential oils from *P. capitarianum* and *P. krukoffii* were less phytotoxic than the synthetic insecticide. The commercial insecticides Deltamethrin (synthetic) and Azadirachtin (plant-based) were respectively 6.93-fold and 3.72-fold more phytotoxic than the *P. capitarianum* oil. Moreover, the *P. krukoffii* was respectively 4.01-fold and 2.12-fold less phytotoxic than Deltamethrin and Azadirachtin.

Table II. Toxicity of the Piper essential oil against Plutellla xylostella.

Essential oil	Bioassay	N	LC_{50} (mg mL^{-1}) (CI‡‡ 95%)	LC_{90} (mg mL^{-1}) (CI‡‡ 95%)	Slope±SE	χ^2	DF
Piper capitarianum	Larvicidal	353	0.21 (0.15-0.29)	1.15 (0.75-2.22)	1.78±0.24	7.01	5
	Ovicidal	356	0.079 (0.068-0.092)	0.25 (0.20-0.33)	2.58±0.23	8.20	5
Piper krukoffi	Larvicidal	382	6.37 (5.10-8.05)	23.38 (16.58-39.83)	2.27±0.29	7.73	5
	Ovicidal	323	2.68 (2.39-3.03)	6.79 (5.54-9.22)	3.18 ± 0.36	1.21	6
Decis®	Larvicidal	337	0.039 (0.035-0.044)	0.14 (0.12-0.18)	2.27±0.13	6.66	5
	Ovicidal	1198	0.066 (0.054-0.079)	1.13 (0.84-1.61)	1.03±0.04	7.20	6
Azamax®	Larvicidal	356	0.033 (0.029-0.038)	0.22 (0.17-0.30)	1.54±0.09	3.68	6
	Ovicidal	1050	0.035 (0.031-0.039)	0.16 (0.13-0.19)	1.94±0.09	6.12	5

N= Number of *Plutellla xylostella*; LC= Lethal Concentration values; CI= Confidence Interval; SE= Standard Error; χ^2=Chi-square; DF= Degree of Freedom.

Table III. Antifeedant activity of the *Piper* essential oil against *Plutella xylostella*.

Essential oil	AC_{50} (mg mL^{-1}) (CI‡‡ 95%)	AC_{90} (mg mL^{-1}) (CI‡‡ 95%)	Slope±SE	χ^2	DF
Piper capiterianum	0.07 (0.06-0.08)	0.24 (0.20-0.30)	2.41±0.16	5.85	6
Piper krukoffi	3.32 (3.12-3.55)	7.41 (6.57-8.63)	3.67±0.25	5.78	6
Azamax®	0.154 (0.13-0.18)	0.87 (0.68-1.19)	1.70±0.11	7.71	5

AC= Antifeedant Concentration values; CI=Confidence Interval; SE=Standard Error; χ2=chi-square. DF=degree of freedom.

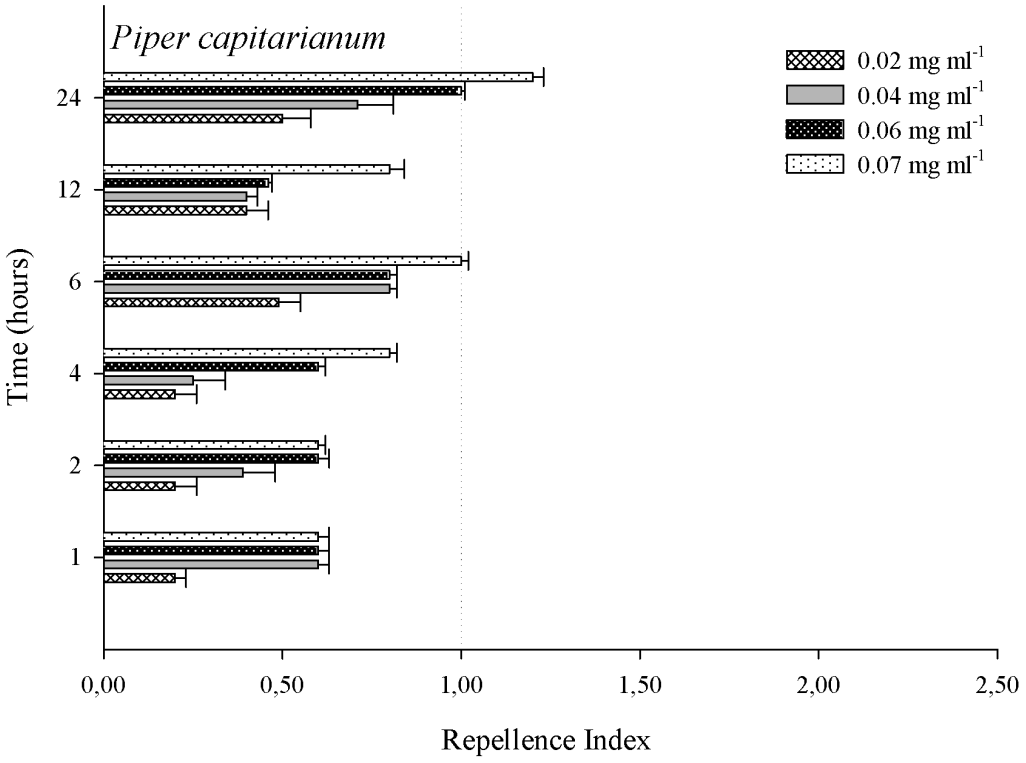


Figure 1. ***P. xylostella*** **larval repellency after exposure to** ***P. capitarianum*** **oil for 24 h.**

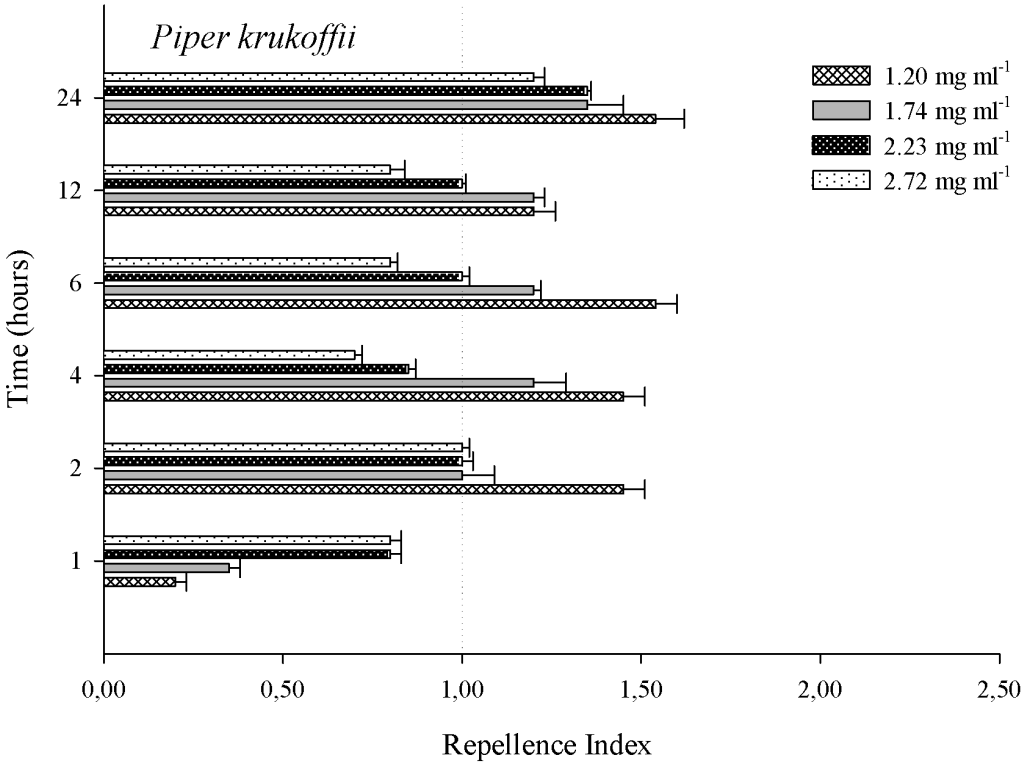


Figure 2. ***P. xylostella*** **larval repellency after exposure to** ***P. krukoffii*** **oil for 24 h.**

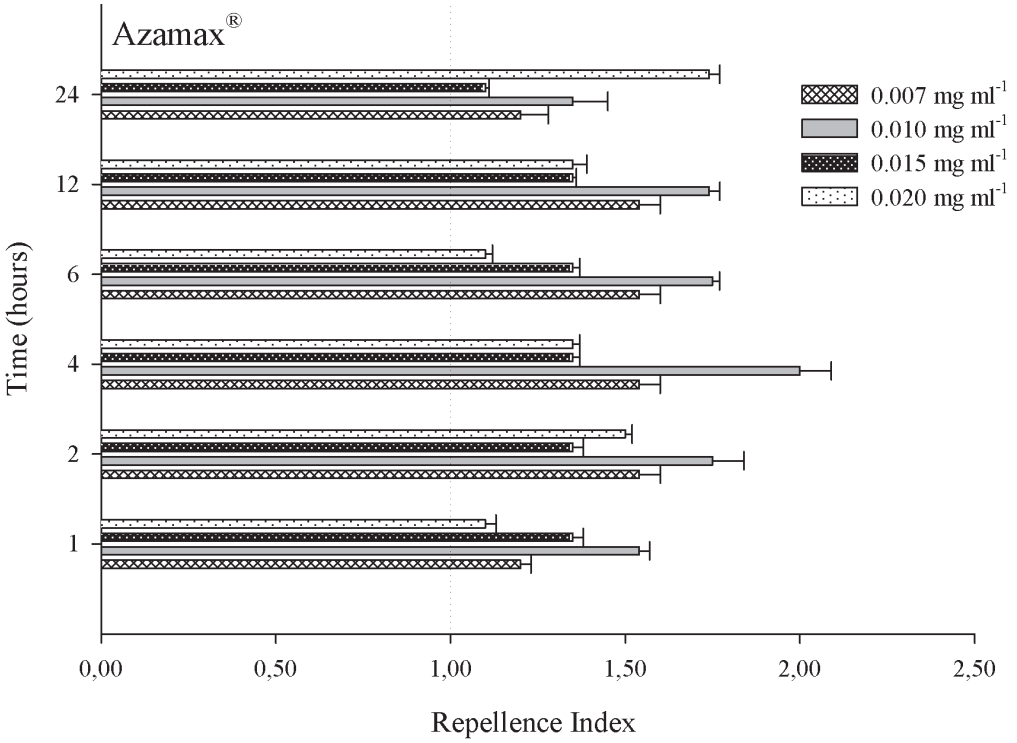
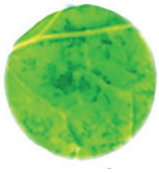


Figure 3. ***P. xylostella*** **larval repellency after exposure to plant-based insecticide Azadirachtin for 24 h.**

Table IV. **Percentage of injury (phytotoxicity) promoted by *Piper* essential oils on collard greens leaf discs.**

	Essential oils		**Positive control**		**Negative control**
	P. krukoffii **%±SE**	***P. capitarianum*** **%±SE**	**Azamax®** **%±SE**	**Decis®** **%±SE**	**Solvent only** **%±SE**
Leaf disc†					
Phytotoxicity	**16.68 ± 1.41**	**9.83 ± 1.21**	**36.58 ± 3.95**	**68.12 ± 1.39**	**0.00 ± 0.00**

SE=Standard Error; The darker area on leaf disc is the injury.

DISCUSSION

The yield for the fresh leaves of *P. krukoffii* oil investigated herein was much lower than that reported by da Silva et al. (2011) for leaves of this species collected in the state of Pará (collection in February, with leaves air dried and three hours of hydrodistillation). This difference may be related to the method and other factors involved in the production and accumulation of essential oils in plants (Lima et al. 2003).

Previous investigations involving GC-MS analyses of the leaf oils of these species reported other chemotypes, such as myristicin/apiole for *P. krukoffii* from the municipality of Parauapebas in the state of Pará, Brazil, and

β-caryophyllene/β-myrcene/α-humulene for *P. capitarianum* from the city of Manaus in the state of Amazonas, Brazil (da Silva et al. 2011). While we found mainly sesquiterpenes in the *P. krukoffii* oil, the *P. capitarianum* oil had similar quantities of monoterpenes and sesquiterpenes. These data diverge from previous reports for this species collected in other localities of the northern Brazil. For instance, oils from *P. krukoffii* collected in the state of Pará and *P. capitarianum* collected in the state of Amazonas were composed predominantly of phenylpropanoids (69.2%) (da Silva et al. 2011) and sesquiterpenes (78.13%) (França 2015), respectively. Methyl eugenol, which was the only phenylpropanoid found in the *P. krukoffii* oil, was identified by da Silva et al. (2011) at a proportion less than 1% in the oil from a sample collected in the state of Pará. In the present investigation, we found two new chemotypes for the species of *Piper* investigated: globulol/4-epi-cis-dihydroagarofuran/γ-muurolene for *P. krukoffii* and *o*-cymene/dehydro-aromadendrene for *P. capitarianum*. Differences in the chemical composition of essential oils from the same species that occur in different localities or even within the same region can be explained by variations in environmental and/or geographic conditions (da Camara et al. 2017).

The insecticidal effects of essential oils from different botanical genera on different development phases of *P. xylostella* have been widely investigated (Chaudhary et al. 2011, Purwatiningsih & Hassan 2012, Reddy et al. 2016, Koundal et al. 2018). Comparing toxicity values, the oil from *P. capitarianum* was more toxic to *P. xylostella* larvae (LC_{50} = 0.21 mg mL^{-1}) than oils from other plant species studied in Brazil and other regions of the world [*Corymbia citriodora* (LC_{50} = 21.53 08 mg mL^{-1}), *Acorus calamus* (LC_{50} = = 0.39 mg mL^{-1}), *Cedrus deodara* (LC_{50} = 1.08 mg mL^{-1}), *Aegle marmelos* (LC_{50} = 8.76 mg mL^{-1}), *Tagetes minuta* (LC_50 = 10.15 mg mL^{-1}), *Murraya koenigii* (LC_{50} = 2.98 mg mL^{-1}), *Curcuma aromatic* (LC_{50} = 1.35 mg mL^{-1}), *Mentha piperita* (LC_{50} = 1.37 mg mL^{-1}), *Mentha spicata* (LC_{50} = 1.86 mg mL^{-1}), *Mentha longifolia* (LC_{50} = 1.06 mg mL^{-1}) and *Cymbopogon flexuosus* (LC_{50} = 1.80 mg mL^{-1})] (Reddy et al. 2016, Filomeno et al. 2017, Koundal et al. 2018).

Few studies have investigated the effects of essential oils from the genus *Piper* on this pest (Sangha et al. 2017). However, other derivates from *Piper* plants have been investigated, such as extracts of different polarities and fixed constituents. A study conducted with hexane extracts from the leaves of different *Piper* species [*P. sarmentosum* (LC_{50} = 2061.29 μg mL^{-1}), *P. interruptum* (LC_{50} = 1328.24 μg mL^{-1}), *P. nigrum* (LC_{50} = 2800.95 μg mL^{-1}) and *P. retrofractum* (LC_{50} = 237.38 μg mL^{-1})] collected in Thailand revealed high toxicity to *P. xylostella* larvae (Kraikrathok et al. 2013). Park (2012) reported toxicity to *P. xylostella* larvae for the hexane extract of *Piper nigrum* (100% mortality at 2.5 mg mL^{-1}) and its major constituents guineensine (LC_{50} = 0.013 mg mL^{-1}), retrofractamide A (LC_{50} = 0.020 mg mL^{-1}), pipercide (LC_{50} = 0.033 mg mL^{-1}) and pellitorine (LC_{50} = 0.046 mg mL^{-}1).

The greater toxicity to the eggs and larvae found for the *P. capitarianum* oil compared to the *P. krukoffii* oil may be explained by qualitative and quantitative chemical differences between the two oils, as demonstrated by GC-MS. Moreover, *P. xylostella* was more susceptible to both oils in the egg phase than in the larval phase. In contrast, Sangha et al. (2017) found that the oil from *P. nigrum* was more toxic to the larval phase than the egg phase of *P. xylostella*. The greater susceptibility of the egg phase in the present investigation may be explained by the physical effect on the eggs. While the oils acted on the larvae through residual contact (affecting target sites after the penetration of the larval tegument), the action on eggs was through

direct contact, as the eggs were immersed in an aqueous solution of the oil, affecting not only target sites in the embryo, but also forming an oily layer on the surface of the egg that served as a barrier impeding the exchange of gases between the embryo and the external environment (Krinski et al. 2018). However, other factors should be considered when evaluating differences in susceptibility between the larval and egg phases of *P. xylostella*, such as the chemical profile of the oil, volatility, degree of lipophilicity and the capacity to form a film on the egg surface.

The comparison of the LC_{50} estimated for the *Piper* oils investigated herein on 3rd instar larvae of *P. xylostella* and values reported for oils from other plants reveals that the oil from *P. capitarianum* is more efficient that oils from *Mentha longifolia* L. Huds., *Mentha piperita* L., *Mentha spicata* L., *Cymbopogon flexuosus* Steud. and *Curcuma aromatica* Salisb (Koundal et al. 2018). In studies on larvicidal action against *P. xylostella,* the toxicity of the essential oil from *Allium tuberosum* L. (LC_{50} = 0.56 µl mL^{-1}) was 2.66-fold lower (Gao et al. 2019) and the toxicity of the essential oil from *Zingiber officinale* Roscoe (LC_{50} = 6176.31 mg L^{-1}) was 29.41-fold lower (Babu et al. 2018) than that found for the *P. capitarianum* oil.

Sangha et al. (2017) found that the oil from *Piper nigrum* L. was toxic to the eggs and larvae of *P. xylostella*, but the comparison of the results reveals that the *P. capitarianum* oil is respectively 160-fold and 34.78-fold more toxic to *P. xylostella* eggs and larvae than the *P. nigrum* oil.

There are few reports in the literature addressing the ovicidal effect of essential oils on *P. xylostella*. However, ovicidal action has been investigated for other lepidopterans. Krinski et al. (2018) reported the ovicidal action of 21 essential oils from species of *Piper* against *Anticarsia gemmatalis* Hübner (Lepidoptera: Eribidae), highlighting the oils from *P. fuligineum* Kunth. and *P. mollicomum* Kunth., which exhibited the same level of toxicity (LC_{50} = 0.4%). Lourenço et al. (2018) found that the viability of eggs from *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) was reduced by up to 80% when exposed to 3.3 µl ml^{-1} of the essential oil from *Siparuna guianensis* Aublet.

The present results on the lethal action of the *P. capitarianum* and *P. krukoffii* oils suggest that these oils affect the larval phase, which causes damage to crops, as well as the egg phase, impeding the development into the larval stage.

The insecticidal potential of essential oils not only causes the death of insects, but also repels, deters feeding, inhibits growth, causes the deformation of pupae and reduces both the longevity and fecundity of insects (Mossa 2016). The antifeedant effect and repellency are important properties of an insecticide for integrated pest management. These properties affect the behavior of the pest, keeping it away from the host and minimizing crop damage (da Camara et al. 2015). The greater antifeedant and repellent properties found for the *P. capitarianum* oil compared to the *P. krukoffii* oil may be attributed to differences in the chemical profile of these oils. These behavioral effects of the *Piper* oils on *P. xylostella* larvae may stem from the monoterpenes and sesquiterpenes that compose the oils, as terpenes are known to have such effects as part of the defense of plants against herbivory (Singh & Sharma 2015, Pichersky & Raguso 2018, Block et al. 2019).

Although a significant number of essential oils from other botanical genera have been evaluated with regards to their effects on *P. xylostella* (Reddy et al. 2016, Wei et al. 2015), this is the first report of the antifeedant effect of oils from species of the genus *Piper* on the larvae of this important pest of cruciferous vegetables. On the other hand, essential oils from other species

of *Piper* have been investigated with regards to their antifeedant effect on other arthropod pests. For instance, the oil from *P. hispidinervum* C. DC. exhibited antifeedant activity against the caterpillars *Spodoptera frugiperda* (Lima et al. 2009) and *S. littoralis* Boisduval (Lepidoptera: Noctuidae) (Andrés et al. 2017) at a concentration of 0.81 mg mL^{-1} and 100 µL cm^2, respectively.

The feeding deterrence found for the *Piper* oils investigated herein, especially the *P. capitarianum* oil, which is composed mainly of monoterpenes and sesquiterpenes, is in agreement with studies conducted by Koul et al. (2008), who state that terpenes are the chemical class with the greatest known antifeedant diversity.

A large number of essential oils extracted from different families have been shown to be highly repellent to arthropod species (Nerio et al. 2010). While investigations evaluating the repellant effect of essential oils on larvae of the order Lepidoptera are scarce, the essential oils from *Zanthoxylum armatum* DC (Kumar et al. 2016), *Tagetes minuta* L., *Mentha spicata* and *Hedychium spicatum* Herm. have been found to be repellent to 3rd instar larvae of *P. xylostella* (Reddy et al. 2016). Although there are no previous reports of the repellent action of essential oils from species of *Piper* against *P. xylostella* larvae, the effects of oils from the genus on other arthropod pests have been investigated. For example, Santana (2018) found that the oil from *P. divaricatum* G. Mey. exhibited repellent activity against the aphid *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphididae) for 24 h. The oil from *P. nigrum* exhibited significant repellency activity against the beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) (Upadhyay & Jaiswal 2007) as well as the cockroach species *Periplaneta americana* L. (Blattaria: Blattidae) and *Blatella germanica* L. (Blattodea: Blattellidae) (Thavara et al. 2007).

Due to their high volatility, essential oils form a vapor barrier that avoids contact between the arthropod and the surface of the host plant (Brown & Hebert 1997). In the present study, greater repellent activity was found for the *P. capitarianum* oil, which suggests a better interaction between the vapor formed by the volatile constituents of the oil and olfactory receptors in the pest (Tyagi 2016).

Phytotoxicity is a concern when formulating new pest control products (Correia & Durigan 2007), as it can cause irreversible damage to the structure and physiology of the host plant (Carvalho et al. 2009). The most common symptom of phytotoxicity is leaf necrosis. The mild and acceptable levels of phytotoxicity respectively found for the *P. capitarianum* and *P. krukoffii* oils to the host plant (*Brassica oleracea* var. acephala) did not cause necrosis to the point of compromising the quality of the product. In contrast, the degrees of necrosis found after the application of the positive controls Deltamethrin and Azadirachtin indicated severe phytotoxicity, compromising the quality of the product to be sold.

Few studies have investigated the phytotoxicity of essential oils from species of *Piper* to host plants of agricultural pests. However, several studies have demonstrated high phytotoxicity of essential oils (Jaramillo-Colorado et al. 2019, Souza Filho et al. 2009, Andrés et al. 2017) and ethanolic extracts (Lustosa et al. 2007, Pukclai & Kato-Noguchi 2011, Huang et al. 2010) from species of this genus on weeds. The phytotoxic effects of essential oils from other plant species on host plants of agricultural pests have been investigated. For instance, oils from *Achillea millefolium* Afan., *Santolina chamaecyparissus* L. and *Tanacetum vulgare* L. presented phytotoxicity at a concentration of

0.8%, with accentuated necrosis on the leaves of the pea (*Pisum sativum* L.), which is a host plant for the aphid *Myzus persicae* (Czerniewicz et al. 2018). In another study, Sertkaya et al. (2010) found no evidence of phytotoxicity of oils from *Origanum onites* L., *Thryptomene spicata* Rye and Trudgen, *Lavandula stoechas* L. and *Mentha spicata* at a concentration of 15 g mL^{-1} on the leaves of different host plants of the pest *Tetranychus urticae* Koch (Acari: Tetranychidae) (tomato, bell pepper, cucumber and eggplant).

The chemical investigation of the *P. capitarianum* and *P. krukoffii* oils using GC-MS enabled the identification of two new chemotypes: globulol/4-epi-*cis*-dihydroagarofuran/γ-muurolene for *P. krukoffii* and *o*-cymene/dehydro-aromadendrene for *P. capitarianum*. This is the first report of the lethal (larvicidal and ovicidal) and sublethal (antifeedant and repellency) effects of the essential oils from *P. krukoffii* and *P. capitarianum* on *P. xylostella* as well as phytotoxicity to the host plant, *Brassicae oleraceae* var. acephala.

The present findings reveal that the *P. capitarianum* oil was more efficient than the *P. xylostella* oil and also when compared to the results of the positive controls (Deltamethrin and Azadirachtin). Moreover, the considerable availability of this plant in the São Francisco/Terra Nova agricultural community of metropolitan Manaus, Brazil, makes it a promising candidate for the preparation of an insecticidal formula containing the essential oil from the leaves. The characterization of the chemical composition of these oils allows them to be used as a standard in the preparation of formulations in laboratories, based on their chemical composition, so that they can be used against the pest. Essential oils have advantages over synthetic insecticides, such as biodegradability, obtainment from renewable sources and generally lower toxicity to mammals. However, due to their high volatility, essential oils are susceptible to degradation by physical (light and temperature) and chemical (air and humidity) agents (Pavela & Sedlák 2018), therefore requiring formulations on a nanometric scale that preserves their physicochemical properties (Pavela et al. 2019). Thus, further studies are needed to evaluate post-application conditions, especially temperature, that may significantly affect the efficacy of *Piper* oils.

The essential oils investigated herein are promising alternatives to synthetic pesticides for the control of *P. xylostella*. As these substances have a natural origin and are generally safer, further studies should be conducted to evaluate possible environmental impacts, especially on non-target organisms, as well as determine the cost-benefit ratio for the formulation of a plant-based insecticide for use in the integrated management of *P. xylostella*. Also, it is necessary to carry out future studies that separately evaluate the compounds found here, as well as mixtures of these, since biotic and abiotic factors can cause qualitatives and quantitatives changes in the chemical composition of the essential oils of the plants.

Acknowledgments

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (PQ-2-302860/2016-9), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (88887.368587/2019-00) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE; grants BCT-0253-1.06/19, APQ-0476-1.06/14, APQ-08601.06/16 and APQ- 10081.06/15).

REFERENCES

ADAMS RP. 2007. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Espectroscopy, 4th ed, Allured Publishing Corporation: Carol Stream, 804 p.

AKHTAR Y, PAGES E, STEVENS A, BRADBURY R, DA CAMARA CAG & ISMAN MB. 2012. Effect of chemical complexity of essential

oils on feeding deterrence in larvae of the cabbage looper. Physiol Entomol 37: 81-91.

ALVEZ A, KOGAN WPHLM, HELFGOTT EES & HANSEN R. 1974. Recomendaciones sobre unificación de los sistemas de avaluacion en ensayos de control de malezas. Ver ALAM 1: 35-38.

ANDRÉS MF, ROSSA GE, CASSEL E, VARGAS RMF, SANTANA O, DÍAZ CE & GONZÁLEZ-COLOMA A. 2017. Biocidal effects of *Piper hispidinervum* (Piperaceae) essential oil and synergism among its main components. Food Chem Toxicol 109: 1086-1092.

BABU GK, DOLMA SK, SHARMA M & REDDY SE. 2018. Chemical composition of essential oil and oleoresins of *Zingiber officinale* and toxicity of extracts/essential oil against diamondback moth (*Plutella xylostella*). Toxin Ver 226-235. doi.org/10.1080/15569543.2018.1491056.

BANDEIRA GN, DA CAMARA CAG, DE MORAES MM, BARROS R, MUHAMMAD S & AKHTAR Y. 2013. Insecticidal activity of *Muntingia calabura* extracts against larvae and pupae of diamondback, *Plutella xylostella* (Lepidoptera, Plutellidae). J King Saud Univ Sci 25: 83-89.

BLOCK AK, VAUGHAN MM, SCHMELZ EA & CHRISTENSEN SA. 2019. Biosynthesis and function of terpenoid defense compounds in maize (*Zea mays*). Planta 249: 21-30.

BROWN M & HEBERT AA. 1997. Insect repellents: an overview. J Am Acad Dermatol 36: 243-249.

BUSTOS G, SILVA G, FISHER S, FIGUEROA I, URBINA A & RODRÍGUEZ JC. 2017. Repelencia de mezclas de aceites esenciales de boldo, laurel chileno, y tepa contra el gorgojo del maíz. Southwest Entomol 42: 551-563.

CARVALHO SJPD, NICOLAI M, FERREIRA RR, FIGUEIRA AVDO & CHRISTOFFOLETI PJ. 2009. Herbicide selectivity by differential metabolism: considerations for reducing crop damages. Sci Agric 66: 136-142.

CASTELO BRANCO M & FRANÇA FH. 2015. Traça das crucíferas, *Plutella xylostella* (L). In: Vilela EF & Zucchi RA (Eds), Pragas introduzidas no Brasil: insetos e ácaros, Piracicaba: FEALQ, p. 516-528.

CHAUDHARY A, SHARMA P, NADDA G, TEWARY DK & SINGH B. 2011. Chemical composition and larvicidal activities of the Himalayan cedar, *Cedrus deodara* essential oil and its fractions against the diamondback moth, *Plutella xylostella*. J Insect Sci 11: 157.

CORREIA NM & DURIGAN JC. 2007. Selectivity of different glyphosate-derived herbicides to soybean RR. Planta Daninha 25: 375-379.

CZERNIEWICZ P, CHRZANOWSKI G, SPRAWKA I & SYTYKIEWICZ H. 2018. Aphicidal activity of selected Asteraceae essential oils and their effect on enzyme activities of the green peach aphid, *Myzus persicae* (Sulzer). Pestic Biochem Phys 145: 84-92.

DA CAMARA CAG, AKHTAR Y, ISMAN MB, SEFFRIN RC & BORN FS. 2015. Repellent activity of essential oils from two species of *Citrus* against *Tetranychus urticae* in the laboratory and greenhouse. Crop Prot 74: 110-115.

DA CAMARA CAG, DE MORAES MM, DE MELO JP & DA SILVA MM. 2017. Chemical composition and acaricidal activity of essential oils from *Croton rhamnifolioides* Pax and Hoffm. in different regions of a Caatinga Biome in Northeastern Brazil. J Essent Oil Bear Pl 20: 1434-1449.

DA SILVA JKR, ANDRADE EHA, KATO MJ, CARREIRA LMM, GUIMARÃES EF & MAIA JGS. 2011. Antioxidant capacity and larvicidal and antifungal activities of essential oils and extracts from *Piper krukoffii*. Nat Prod Commun 6: 1361-1366.

DE MELO JPR & DA CAMARA CAG. 2019. Produto formulado a base de óleos essenciais de *Citrus reticulata* para o controle da traça das crucíferas (*Plutella xylostella*). BR Patent Application Nº 1020150264488. Instituto Nacional da Propriedade Industrial.

FILOMENO CA, BARBOSA LCA, TEIXEIRA RR, PINHEIRO AL, SÁ EF, SILVA EMP & PICANÇO MC. 2017. *Corymbia* spp. and *Eucalyptus* spp. essential oils have insecticidal activity against *Plutella xylostella*. Ind Crop Prod 109: 374-383.

FINNEY DJ. 1971. Probit Analysis. 3rd ed, New York: Cambridge University Press, 333 p.

FRANÇA LP. 2015. Avaliação da atividade larvicida de extratos e óleo essencial de *Piper capitarianum Yunck*, 1966 (Piperaceae) sobre *Aedes aegypti* Linnaeus, 1762 e *Anopheles sp* (Culicidae) em laboratório. Universidade Federal do Amazonas, Manaus, Dissertação (Mestrado em Biotecnologia), 107 p. (Unpublished).

GAO Q, SONG L, SUN J, CAO HQ, WANG L, LIN H & TANG F. 2019. Repellent action and contact toxicity mechanisms of the essential oil extracted from Chinese chive against *Plutella xylostella* larvae. Arch Insect Biochem 100: e21509.

GUIMARÃES EF, CARVALHO-SILVA M, MONTEIRO D, MEDEIROS ES & QUEIROZ GA. 2015. Lista de Espécies da Flora do Brasil. fromhttp://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB190

HUANG H, MORGAN CM, ASOLKAR RN, KOIVUNEN ME & MARRONE PG. 2010. Phytotoxicity of sarmentine isolated from long pepper (*Piper longum*) fruit. J Agr Food Chem 58: 9994-1000.

JARAMILLO-COLORADO BE, PINO-BENITEZ N & GONZÁLEZ-COLOMA A. 2019. Volatile composition and biocidal (antifeedant and phytotoxic) activity of the essential oils of four Piperaceae species from Choco-Colombia. Ind Crops Prod 138: 111463.

KOUL O, WALIA S & DHALIWAL GS. 2008. Essential oils as green pesticides: potential and constraints. Biopestic Int 4: 63-84.

KOUNDAL R, DOLMA SK, CHAND G, AGNIHOTRI VK & REDDY SE. 2018. Chemical composition and insecticidal properties of essential oils against diamondback moth (*Plutella xylostella* L.). Toxin Rev 371-381. doi.org/10.1080/15569543.2018.1536668.

KRAIKRATHOK C, NGAMSAENGI S, BULLANGPOTI V, PLUEMPANUPAT W & KOUL O. 2013. Bio efficacy of some Piperaceae plant extracts against *Plutella xylostella* L. (Lepidoptera: Plutellidae). Commun Agric Appl Biol Sci 78: 305-309.

KRINSKI D, FOERSTER LA & DESCHAMPS C. 2018. Ovicidal effect of the essential oils from 18 Brazilian Piper species: controlling *Anticarsia gemmatalis* (Lepidoptera, Erebidae) at the initial stage of development. Acta Sci 40: e35273.

KUMAR V, REDDY SE, CHAUHAN U, KUMAR N & SINGH B. 2016. Chemical composition and larvicidal activity of *Zanthoxylum armatum* against diamondback moth, *Plutella xylostella*. Nat Prod Res 30: 689-692.

LEWINSOHN TM & PRADO PI. 2005. How many species are there in Brazil?. Conserv Biol 19: 619-624.

LIMA HRP, KAPLAN MAC & CRUZ ADM. 2003. Influência dos fatores abióticos na produção e variabilidade de terpenóides em plantas. FLORAM 10: 71-77.

LIMA NETO JE, AMARAL MH, SIQUEIRA HA, BARROS R & SILVA PA. 2016. Resistance monitoring of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) to risk-reduced insecticides and cross resistance to spinetoram. Phytoparasitica 44: 631-640.

LIMA RK, CARDOSO MG, MORAES JC, MELO BA, RODRIGUES VG & GUIMARÃES PL. 2009. Atividade inseticida do óleo essencial de pimenta longa (*Piper hispidinervum* C. DC.) sobre lagarta-do-cartucho do milho *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae). Acta Amaz 39: 377-382.

LOBO AP, DA CAMARA CAG, DE MELO JPR & DE MORAES MM. 2019. Chemical composition and repellent activity of essential oils from the leaves of *Cinnamomum zeylanicum* and *Eugenia uniflora* against *Diaphania hyalinata* L. Lepidoptera: Crambidae). J Plant Dis Protect 126: 79-87.

LOURENÇO AM ET AL. 2018. Essential oil of *Siparuna guianensis* as an alternative tool for improved lepidopteran control and resistance management practices. Sci Rep 8: 7215.

LUSTOSA FLF, OLIVEIRA SCC & ROMEIRO LA. 2007. Efeito alelopático de extrato aquoso de *Piper aduncum* L. e *Piper tectoniifolium* Kunth na germinação e crescimento de *Lactuca sativa* L. Rev Bras Biocienc 5: 849-851.

MAIA JGS & ANDRADE EHA. 2009. Database of the Amazon aromatic plants and their essential oils. Quim Nova 32: 595-622.

MAZZONETTO F & VENDRAMIM JD. 2003. Efeito de pós de origem vegetal sobre Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae) em feijão armazenado. Neotrop Entomol 32: 145-149.

MOHAN M, SUSHIL SN, SELVAKUMAR G, BHATT JC, GUJAR GT & GUPTA HS. 2009. Differential toxicity of *Bacillus thuringiensis* strains and their crystal toxins against high-altitude Himalayan populations of diamondback moth, *Plutella xylostella* L. Pest Manag Sci 65: 27-33.

MORAES LAS & MARINHO-PRADO JS. 2016. Plantas com atividade inseticida. In: Halfeld-Vieira et al. (Eds), Defensivos agrícolas naturais: uso e perspectivas, Jaguariúna: Embrapa Meio Ambiente, p. 542-593.

MOSSA ATH. 2016. Green pesticides: Essential oils as biopesticides in insect-pest management. J Environ Sci Technol 9: 354.

NERIO LS, OLIVERO-VERBEL J & STASHENKO E. 2010. Repellent activity of essential oils: a review. Bioresour Technol 101: 372-378.

OLIVEIRA AC, SIQUEIRA HAA, OLIVEIRA JV, SILVA JE & MICHEREFF FILHO M. 2011. Resistance of Brazilian diamondback moth populations to insecticides. Sci Agric 68: 154-159.

PARK IK. 2012. Larvicidal activity of constituents identified in *Piper nigrum* L. fruit against the diamondback moth, *Plutella xylostella*. Korean J Appl Entomol 51: 149-152.

PAVELA R & SEDLÁK P. 2018. Post-application temperature as a factor influencing the insecticidal activity of essential oil from *Thymus vulgaris*. Ind Crops Prod 113: 46-49.

PAVELA R ET AL. 2019. Microemulsions for delivery of Apiaceae essential oils—Towards highly effective and eco-friendly mosquito larvicides?. Ind Crops Prod 129: 631-640.

PEREIRA ACRL, OLIVEIRA JV, GONDIM JUNIOR MGC & DA CAMARA CA G. 2008. Atividade inseticida de óleos essenciais e fixos sobre *Callosobruchus maculatus* (FABR., 1775)

(Coleoptera: Bruchidae) em grãos de caupi [*Vigna unguiculata* (L.) WALP.]. Cienc Agrotec 32: 717-724.

PICHERSKY E & RAGUSO RA. 2018. Why do plants produce so many terpenoid compounds?. New Phytol 220: 692-702.

PITON LP, TURCHEN LM, BUTNARIU AR & PEREIRA MJB. 2014. Natural insecticide based-leaves extract of *Piper aduncum* (Piperaceae) in the control of stink bug brown soybean. Cienc Rural 44: 1915-1920.

PUKCLAI P & KATO-NOGUCHI H. 2011. Allelopathic Activity of *Piper sarmentosum* Roxb. Asian J Plant Sci 10: 147-152.

PURWATININGSIH HN & HASSAN E. 2012. Efficacy of *Leptospermum petersonii* oil, on *Plutella xylostella*, and its parasitoid, *Trichogramma pretiosum*. J Econ Entomol 105: 1379-1384.

QUIJANO-ABRIL MA, CALLEJAS-POSADA R & MIRANDA-ESQUIVEL DR. 2006. Areas of endemism and distribution patterns for Neotropical Piper species (Piperaceae). J Biogeogr 33: 1266-1278.

REDDY SE, DOLMA SK, KOUNDAL R & SINGH B. 2016. Chemical composition and insecticidal activities of essential oils against diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). Nat Prod Res 30: 1834-1838.

RIBEIRO LM, SIQUEIRA HA, WANDERLEY-TEIXEIRA V, FERREIRA HN, SILVA WM, SILVA JE & TEIXEIRA AA. 2017. Field resistance of Brazilian *Plutella xylostella* to diamides is not metabolism-mediated. Crop Prot 93: 82-88.

ROBERTSON JL, JONES MM, OLGUIN E & ALBERTS B. 2017. Bioassays with arthropods. Boca Raton: CRC press, 194 p.

SANGHA JS, ASTATKIE T & CUTLER GC. 2017. Ovicidal, larvicidal, and behavioural effects of some plant essential oils on diamondback moth (Lepidoptera: Plutellidae). Can Entomol 149: 639-648.

SANTANA HT, TRINDADE FTT, STABELI RGS, SILVA AAE, MILITÃO JSTL & FACUNDO VA. 2015. Essential oils of leaves of *Piper* species display larvicidal activity against the dengue vector, *Aedes aegypti* (Diptera: Culicidae). Rev Bras Plantas Med 17: 105-111.

SANTANA MF. 2018. Toxicidade, repelência, taxa instantânea e efeito de óleos essenciais sobre *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphidadae) e seu inimigo natural *Aphidius sp*. (Hymenoptera: Braconidae). Tese de Doutorado. UFRPE: Recife, 64 p.

SAS. 2002. SAS User's Guide: Statistics Version 9.0. Cary: SAS Institute.

SERTKAYA E, KAYA K & SOYLU S. 2010. Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.) (Acarina: Tetranychidae). Ind Crops Prod 31: 107-112.

SILVA CGV, OLIVEIRA JCS & DA CAMARA CAG. 2018. Insecticidal activity of the ethanolic extract from *Croton* species against *Plutella xylostella* L. (Lepidoptera: Plutellidae). Rev Fac Nac Agron Medellin 71: 8543-8551.

SINGH B & SHARMA RA. 2015. Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. Biotech 5: 129-151.

SOUZA FILHO APDS, VASCONCELOS MAMD, ZOGHBI MDGB & CUNHA RL. 2009. Potentially allelopathic effects of the essential oils of *Piper hispidinervium* C. DC. and *Pogostemon heyneanus* (Benth) on weeds. Acta Amaz 39: 389-395.

THAVARA U, TAWATSIN A, BHAKDEENUAN P, WONGSINKONGMAN P, BOONRUAD T, BANSIDDHI J & MULLA MS. 2007. Repellent activity of essential oils against cockroaches (Dictyoptera: Blattidae, Blattellidae, and Blaberidae) in Thailand. Southeast Asian J Trop Med Public Health 38: 663-673.

TORRES AL, BOIÇA JÚNIOR AL, MEDEIROS CAM & BARROS R. 2006. Efeito de extratos aquosos de *Azadirachta indica*, *Melia azedarach* e *Aspidosperma pyrifolium* no desenvolvimento e oviposição de *Plutella xylostella*. Bragantia 65: 447-457.

TYAGI BK. 2016. Advances in vector mosquito control technologies, with particular reference to herbal products. In: VIJAY & REJI (Eds), Herbal insecticides, repellents and biomedicines: effectiveness and commercialization, New Delhi: Springer, p. 1-9.

UPADHYAY RK & JAISWAL G. 2007. Evaluation of biological activities of *Piper nigrum* oil against *Tribolium castaneum*. Bull Insectology 60: 57-61.

VAN DEN DOOL H & KRATZ PH. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr A 11: 463-471.

WEI H, LIU J, LI B, ZHAN Z, CHEN Y, TIAN H, LIN S & GU X. 2015. The toxicity and physiological effect of essential oil from *Chenopodium ambrosioides* against the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Crop Prot 76: 68-74.

ZAGO HB, BARROS R, TORRES JB & PRATISSOLI D. 2010. Egg distribution of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and the parasitism by *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Neotrop Entomol 39: 241-247.

ZALUCKI MP, SHABBIR A, SILVA R, ADAMSON D, SHU-SHENG L & FURLONG MJ. 2012. Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera: Plutellidae): just how long is a piece of string?. J Econ Entomol 105: 1115-1129.

SUPPLEMENTARY MATERIAL

Figure S1

How to cite

SANTANA MLG, DE MELO JPR, DA CAMARA CAG, DE MORAES MM, DE ARAUJO CA, DE VASCONCELOS GJN, PEREIRA MRS & ZARTMAN CE. 2022. Lethal and sublethal effects of essential oils from *Piper capitarianum* Yunck and *Piper krukoffii* Yunck on *Plutella xylostella* L. An Acad Bras Cienc 94: e20200072. DOI 10.1590/0001-3765202220200072.

Manuscript received on January 16, 2020; accepted for publication on May 06, 2020

MILENA L.G. SANTANA[1]
https://orcid.org/ 0000-0003-2303-568X

JOÃO P.R. DE MELO[2]
https://orcid.org/0000-0002-1301-7763

CLÁUDIO A.G. DA CAMARA[1,2]
https://orcid.org/0000-0001-8508-1230

MARCÍLIO M. DE MORAES[2]
https://orcid.org/0000-0002-7597-6775

CAROLINA A. DE ARAUJO[2]
https://orcid.org/ 0000-0002-4293-1255

GERALDO J.N. DE VASCONCELOS[3]
https://orcid.org/0000-0002-2581-9931

MARTA R.S. PEREIRA[4]
https://orcid.org/0000-0001-7236-2383

CHARLES E. ZARTMAN[4]
https://orcid.org/0000-0001-8481-9782

[1]Universidade Federal Rural de Pernambuco, Laboratório de Investigação Química de Inseticidas Naturais, Departamento de Agronomia, Rua Dom Manoel de Medeiros, s/n, 52171-030 Recife, PE, Brazil

[2]Universidade Federal Rural de Pernambuco, Laboratório de Produtos Naturais Bioativos, Departamento de Química, Rua Dom Manoel de Medeiros, s/n, 52171-030 Recife, PE, Brazil

[3]Universidade Federal do Amazonas, Campus ICET, Rua Nossa Senhora do Rosário, 3863, Tiradentes, 69103-128 Itacoatiara, AM, Brazil

[4]Instituto Nacional de Pesquisa da Amazônia, Coordenação de Pesquisas em Botânica, Av. André Araújo, 2936, Petrópolis, 69060-001 Manaus, AM, Brazil

Correspondence to: **Cláudio Camara**
E-mail: claudio_agc@hotmail.com

Author contributions

Milena L.G. Santana, João P.R. de Melo, Cláudio A.G. da Câmara, conceived study and experimental design. Milena L.G. Santana, João P.R. de Melo, Cláudio A.G. da Câmara, Carolina A. de Araújo, Geraldo J.N. de Vasconcelos, Marta R.S. Pereira and Charles E. Zartman conducted the experiments. João P. R. de Melo, Marcílio M. de Moraes, Carolina A. de Araújo and Cláudio A. G. da Câmara analysed the data. Milena L. G. Santana, João P. R. de Melo and Cláudio A. G. da Câmara secured funding. Milena L. G. Santana, João P. R. de Melo and Cláudio A. G. da Câmara wrote the manuscript. All authors read and approved the manuscript.