

Volatile chemical composition of Colombian *Piper gorgonillense* Trel. & Yunck. essential oil and its repellent and fumigant activity against *Tribolium castaneum* Herbst

Composición química volátil del aceite esencial colombiano de *Piper gorgonillense* Trel. & Yunck. y su actividad repelente y fumigante contra el *Tribolium castaneum* Herbst



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Piper gorgonillense Trel. & Yunck.

Source: Herbarium of Universidad Tecnológica del Choco Diego Luis Córdoba (No. 11990) herbarium of the Universidad de Antioquia (No-493853)

ABSTRACT

Essential oils (EOs) are mixtures of volatile organic compounds, mostly terpenes, from the secondary metabolism of plants. These oils exert various activities on insects that damage crops and cause losses worldwide for the economy and agriculture. *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is one of the main pests causing the loss of a large amount of stored food. The objective of this study was to evaluate the volatile chemical composition of the essential oil from *Piper gorgonillense* Trel. & Yunck. and its repellent and fumigant activity on *T. castaneum*. The volatile composition was determined with gas chromatography coupled to mass spectrometry. The majority compounds found in the EO were β -caryophyllene (28.7%), α -copaene (13.5%), and δ -cadinene (7.3%). The repellency percentages obtained were 78 and 90% at a concentration of 1% with exposure times of 48 and 72 hours, respectively. The fumigant activity refers to the insecticidal action that an essential oil vapor can have without coming into direct contact with insects; this was 100% at a concentration of EO 350 $\mu\text{g mL}^{-1}$. The results showed that the *P. gorgonillense* EO had repellent and insecticidal properties for the biological control of *T. castaneum*.

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Additional keywords: essential oil crops; repellency; mortality; terpenes; gas chromatography.

RESUMEN

Los aceites esenciales (AEs) son mezclas de compuestos orgánicos volátiles, en su mayoría terpenos, provenientes del metabolismo secundario de las plantas. Estos tienen diversas actividades sobre insectos que dañan las cosechas y producen grandes pérdidas en la economía y la agricultura mundial. El *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), es una de las principales plagas causante de la pérdida de gran cantidad de alimentos almacenados. El objetivo de este estudio fue evaluar la composición química volátil del aceite esencial de *Piper gorgonillense* Trel. & Yunck. y su actividad repelente y fumigante sobre el *T. castaneum*. La composición volátil se determinó empleando cromatografía de gases acoplada a espectrometría de masas. Los compuestos mayoritarios encontrados en el AE fueron β -cariofileno (28.7%), α -copaeno (13.5%), y δ -cadinene (7.3%). Los porcentajes de repelencia obtenidos fueron de 78 y 90% a una concentración de 1% con tiempos de exposición de 48 y 72 horas, respectivamente. La actividad fumigante hace referencia a la acción insecticida que tiene el vapor del aceite esencial sin tener contacto directo con los insectos, este fue de 100% a una concentración de 350 $\mu\text{g mL}^{-1}$. Los resultados mostraron que el AE de *P. gorgonillense* presentó propiedades repelentes e insecticidas para el control biológico de *T. castaneum*.

Palabras clave adicionales: aceite esencial de cultivos; repelencia; mortalidad; terpenos; cromatografía de gases.

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INTRODUCTION

Piper gorgonillense Trel. & Yunck is an accepted name for a species in the *Piper* genus (*Piperaceae* family) (The Plant List, 2013). It is a bush native to Colombia that can reach 3 m, which has been found in the canyon and valley of Bajo Cauca, valley of Magdalena Medio, the jungle valley of Atrato and Uraba (0-500 m altitude), eastern slope of the Central Mountain Range or *Cordillera Central* (0-1,000 m), and the Nariño department (Idárraga and Callejas, 2011). Plants of the *Piper* genus have shown various biological activities, such as anti-inflammatory (Rengifo *et al.*; 2019), phytotoxic, antifungal (Parra *et al.*, 2019), antimicrobial (Gamboa *et al.*, 2018), repellent and insecticidal properties (Jaramillo-Colorado *et al.*, 2019a; Chaubey; 2011), among others. Approximately, two hundred and fifty species of insects attack grains and their products during storage, of which around twenty are of paramount importance (Opit *et al.*, 2012); among these, one of the most damaging is the brown flour beetle, whose common name comes from its color and its habits of infesting flour, while its scientific name is *Tribolium castaneum* Herbst. This beetle belongs to the Insect class, *Coleoptera* order and *Tenebrionidae* family (Athanasios *et al.*, 2016). It is one of the most important stored product blights found in

homes and grocery stores. It has an Indo-Australian origin and currently occurs worldwide in warmer climates. *T. castaneum* Herbst is a secondary pest that generally feeds on stored cereals and grains (Adamski *et al.*, 2019).

To minimize these losses, chemical pesticides are normally used, where mixtures of insecticides and fungicides are utilized in order to protect the seeds during storage; however, chemicals can generate seed toxicity and lead to resistance problems, environmental contamination, and food residues (Daglish *et al.*, 2015). Several authors have reported the use of essential oils as a non-chemical option for the management of these insect pests (Singh and Pandey, 2018; Andrés *et al.*, 2017). EOs are obtained from aromatic plants and are made up of different volatile organic compounds, so their biological activities will rely on their composition and the interaction between their components (Kendra *et al.*, 2017).

The objective of this study was to determine the volatile chemical composition of the essential oil from *P. gorgonillense* by assessing its repellent and fumigant activity against *T. castaneum*.

MATERIALS AND METHODS

Reagents and chemicals

Anhydrous Na_2SO_4 was acquired from Merck (Darmstadt, Germany). Dichloromethane, acetone, and hexane were acquired from AppliChem Panreac (Darmstadt, Germany), along with DEET (Dr. Ehrenstorfer, Germany) and pirimiphos methyl (Syngenta, Colombia). Filter paper was purchased from GE Healthcare (Hangzhou, China).

Vegetal material

Fresh *Piper gorgonillense* Trel. & Yunck. leaves and stems were collected in a rural area of Quibdo (Colombia) in July 2018. The collection site is characterized as a growing area and is also vulnerable to flooding. Taxonomic identification was done at the Herbarium Universidad de Antioquia. The control leaves of each plant are archived as a permanent sample at the Herbarium (No-493853).

Extraction of the essential oil

The extraction was performed using the hydrodistillation technique, according to Jaramillo-Colorado *et al.* (2012). 500 g of leaves and stems from *P. gorgonillense* were used, finely chopped and submerged in boiling water with conventional heating for 2 h. The EO was separated with decantation, and then anhydrous Na_2SO_4 was added to the oil. One EO aliquot (30 μL) was diluted in one mL of dichloromethane for the gas chromatography analysis (Jaramillo *et al.*, 2020).

Chromatography analysis

The EO was analyzed in an Agilent Technologies GC-MS system model 7890A Network GC coupled to a mass selective detector model 5975 (Palo Alto, CA) equipped with a split/split-less injection port (230°C, split ratio 20:1). The mass spectra were obtained with electron-impact ionization at 70 eV energy. The GC conditions were as follows: A HP-5MS capillary column (30 m \times 0.25 mm id \times 0.25 μm df) with 5% phenyl-poly (methyl siloxane) stationary phase was used for the separation of mixtures. The initial oven temperature was 50°C for 2 min, increased at a rate of 5°C min⁻¹ up to 250°C (5 min). The carrier gas was

helium, with an inlet pressure at the head of the column of 12.667 psi at a rate of 1.172 mL min⁻¹, at 50°C. The mass spectra and Kovàts retention indexes were compared to those reported in the literature (Adams, 2007).

Insects and bioassays

T. castaneum adults were collected 7 d after hatching. Bioassays were carried out in the dark in incubators at 28-30°C and 70-80% relative humidity at the Agrochemical Research Laboratory of the University of Cartagena. Oat (*Avena sativa*) was used to feed *T. castaneum*.

Repellent activity

The repellent property of *P. gorgonillense* EO was analyzed on adult specimens of *T. castaneum* using the area preference method. The *P. gorgonillense* EO was dissolved in acetone after preparing five solutions (1, 0.1, 0.01, 0.001, and 0.0001%). A filter paper of 9-cm diameter sheet was cut in half, and 500 μL of each concentration was applied separately to one of the halves of the filter paper as evenly as possible with a micropipette. The other half (control) was treated with 500 μL of acetone. DEET (N, N-diethyltoluamide), a commercial repellent, was the positive control.

The treated and control media disks were dried at room temperature for 10 min to allow the solvent to evaporate. The treated and untreated halves were attached using adhesive tape and placed on Petri dishes. Twenty adult specimens (5 to 7 d old) of *T. castaneum* were placed, one by one, in the center of each filter paper disc with the help of tweezers. The dishes were then covered and, after approximately 5 min, transferred to an incubator at room temperature (Jaramillo-Colorado *et al.*, 2019b). Four replicates were used for each concentration. The weevil preference was measured for each Petri dish at 2 and 4 h of exposure.

The following equation was used to determine the percentage of repellency (PR), following the parameters identified by Jaramillo-Colorado *et al.* (2012) (Eq. 1):

$$\text{PR} = [(N_c - N_t) / (N_c + N_t)] \times 100 \quad (1)$$

where, N_c is the number of insects in the control area (acetone), and N_t is the number of insects in the treated area (EO + acetone).

Fumigant activity

Fumigant activity was performed according to Jaramillo-Colorado *et al.* (2019b). The toxic effect of *P. gorgonillense* EO and terpenes were assayed on *T. castaneum*. Filter paper discs (Whatman No. 1, 2-cm in diameter) were laid down at the bottom of Petri dish covers (90 x 15 mm), which were impregnated with oil at doses calculated to provide equivalent fumigant concentrations of 500, 350, 250, 150, 50 $\mu\text{g mL}^{-1}$ air of oil, respectively. Twenty adult insects (1 to 10 days old) were introduced and tightly capped (replicated four times for each concentration). Pirilan, a commercial pesticide containing methyl pirimiphos (Syngenta, Colombia) (organophosphorus pesticide, 300 $\mu\text{g L}^{-1}$ air) as an active ingredient, was used as the positive control. The mortality percentage was determined after 24 h from the start of exposure.

The percentage of mortality was calculated using Equation 2:

$$\% \text{ Mortality} = [(MT - MC) / (100 - MC)] * 100 \quad (2)$$

where, *MT* and *MC* are the number of dead insects in the treated and control areas, respectively.

Statistical analysis

The results were converted into fumigant percentage and analyzed with ANOVA and Student *t* tests. The mortality rates were calculated using the statistical formulas of Abbott and *Probit* to determine the LC_{50} , chi-square values and related parameters. Biostat, a statistical program (Analyst Soft Robust Business Solutions, BioStat v 2009), was used with a confidence level of 5%. Four replicates for each analysis were performed.

RESULTS AND DISCUSSION

The hydrodistillation technique isolated the essential oil from *P. gorgonillense*, and a yield of 0.14% was obtained, which, compared to other studies of this genus, was lower than the 0.21% seen with extraction with hydrodistillation of *Piper corcovadensis* EO (Miq.) (da Silva *et al.*, 2016). Similarly, a study in Brazil obtained an essential oil yield of 0.44% with hydrodistillation extraction from *Piper cubataonum* (Santos *et al.*, 2014); another study where oil was extracted with steam distillation from *Piper hispidinervum* saw

0.95% (Sauter *et al.*, 2012); finally, the essential oil yield from *P. hispidinervum* obtained with steam distillation reached 0.632% in a study by Andrés *et al.* (2017). The volatile chemical composition of EO is shown in table 1. Figure 1 shows the chemical structure of the main compounds of the *P. gorgonillense* essential oil.

After an intense bibliographic search, it was confirmed that this study is the first report on the volatile chemical composition of *P. gorgonillense* essential oil. The main compound found in this oil was β -caryophyllene, which is a cyclic sesquiterpene; these types of compounds are characterized by their unique structure, which has a bicyclo undecane ring system in the essential oil of a large variety of plant species. This compound is characterized as being volatile and aromatic with low solubility. It is recognized for deploying effective biological activities, such as anti-inflammatory, antiviral, anticarcinogenic, and antimutagenic, among other properties (De Oliveira-Tintino *et al.*, 2018, Wu *et al.*, 2018; Parisotto *et al.*, 2020).

The chemical composition in this study differed slightly from the results obtained by other species belonging to the *Piperaceae* family, such as essential oil from *Piper guineense* with the following main components: β -sesquiphellandrene (20.9%), linalool (6.1%), limonene (5.8%), *Z*- β -bisabolene (5.4%) and α -pinene (5.3%) (Oyemitan *et al.*, 2015); some of these compounds were found in the *Piper gorgonillense* EO but at a much lower proportion, for example, linalool (0.6%), limonene (0.2%) and α -pinene (0.2%). On the other hand, the oils extracted from *Piper dilatatum* had apiol (79.0%), *trans*-caryophyllene (8.3%), spathulenol (4.2%) and γ -cadinene (1.7%); *Piper divaricatum* EO had eugenol (37.5%), methyl eugenol (36.3%), γ -elemene (10.7%), asarone (4.6%) and *trans*-caryophyllene (3.0%); *Piper aff. hispidum* contained δ -3-carene (9.6%), *p*-cymene (10.9%), limonene (17.2%), elemol (14.1), γ -elemene (7.3%) and β -eudesmol (5.7%), whose composition markedly differs from that of *Piper gorgonillense* (Jaramillo-Colorado *et al.*, 2019a).

Whereas, the *Piper tuberculatum* EO is composed of sesquiterpenes and their oxygenated derivatives, such as, (-)-spathulenol (11.37%), α -farnesene (6.22%), (-)-humulene epoxide II (6.04%), β -eudesmol (4.36%), 2-tridecanone (4.27%), 2-pentadecanone (4.06%), ledane (3.62%), (E,E)-farnesyl-acetone (3.55%), β -cedrenoxide (2.96%), α -cadinol (2.86%),

Table 1. Volatile chemical composition of essential oil from Colombian *P. gorgonillense*.

No	Compounds ^a	RI (HP-5) ^b experimental	RI (HP-5) theoretical	Relative area ^c (%)
1	α -Pinene	935	939	0.2
2	Camphene	950	954	0.3
3	β -Pinene	968	979	0.5
4	Limonene	1030	1029	0.2
5	Linalool	1100	1096	0.6
6	Anisole	1225	1232	0.2
7	δ -Elemene	1332	1335	0.6
8	1,5,5-Trimethyl-6-methylene-cyclohexene	1340	1338	1.8
9	α -Cubebene	1342	1345	1.3
10	α -Copaene	1368	1374	13.5
11	β -Bourbonene	1380	1387	2.0
12	β -Elemene	1385	1389	0.9
13	α -Gurjunene	1410	1409	2.7
14	β -Caryophyllene	1425	1419	28.7
15	β -Gurjunene (β -Calarene)	1430	1433	3.2
16	Aromadendrene	1444	1441	2.3
17	Humulene	1450	1454	2.1
18	Allo-Aromadendrene	1462	1460	1.3
19	γ -Muurolene	1474	1479	1.6
10	Germacrene D	1480	1484	1.0
21	<i>trans</i> -Muurolo-4(14),5-diene	1496	1493	2.1
22	α -Muurolene	1500	1500	2.7
23	Epi-bicyclosesquiphellandrene	1515	1521	1.9
24	δ -Cadinene	1520	1523	7.3
25	α -Cadinene	1530	1538	1.1
26	α -Calacorene	1538	1544	1.7
27	Spathulenol	1570	1578	2.5
28	Caryophyllene oxide	1581	1583	0.9
29	Globulol	1588	1590	1.7
30	Guaiol	1596	1600	1.0
31	<i>trans</i> - β -Elemenone	1600	1601	0.9
32	Cubenol <1-epi->	1622	1628	0.5
33	Cadin-4-en-7-ol <cis->	1628	1636	0.8
34	<i>tau</i> -Cadinol (epi- α -cadinol)	1636	1640	3.6
35	Epoxide-allo aromadendrene	1639	1641	0.5
36	α -Cadinol (<i>tau</i> -Muurolol)	1645	1642	2.1
37	Cubenol	1650	1646	1.4
38	Patchouli alcohol	1656	1658	1.0
39	Eudesm-7(11)-en-4-ol (Juniper camphor)	1720	1700	0.8
40	Phytol	1932	1943	0.5

^a Identification made with mass spectrometry (EI: electron impact ionization, 70 eV; peak matching >90%) and LRIs. Spectral databases wiley8, NIST08. ^b Experimentally RI on the HP-5, averages of three independent extractions. ^c RI –Linear retention indices relative to C₇–C₃₀ n-alkanes. Forty compounds were identified, where sesquiterpenic type compounds predominated. The main components were α -copaene (13.5%), β -caryophyllene (28.7%), δ -cadinene (7.3%) and *tau*-cadinol (3.6%).

dibutylphthalate (2.84%), (1RS, 2SR) -2-hydroxy-2,4,4-trimethyl-3- (3-methyl-3-butenylidene) cyclopentyl methyl ketone (2.68%), 4-norpyridoxol (2.26%), epiglobulol (2.14%) and 8-acetyl-3, 3-epoxymethane-6,6,7-trimethylbicyclo [5,1,0] octan-2-one (2.11 %) (Ordaz *et al.*, 2011). In addition, the chemical identification of *Piper marginatum* had a higher percentage of sesquiterpenes; the main compounds included 3,4-methylenedioxypropophenone (22.90%), δ -3-carene (10.19%), trans-caryophyllene (9.67%) and spathulenol (6.89%) (Gomes-Macêdo *et al.*, 2020). However, the characterization of the essential oil from *Piper aleyreanum* saw a slight similarity in the composition of *P. gorgonillense* because sesquiterpenoids prevailed in *P. aleyreanum*, with β -elemene (16.3%), bicyclogermacrene (9.2%), δ -elemene (8.2%), germacrene D (6.9%), β -caryophyllene (6.2%) and spathulenol (5.2 %) as the main components; as for the *Piper anonifolium* oil, the composition was dominated by selin-11-en-4- α -ol (20.0%), β -selinene (12.7%), α -selinene (11.9%) and α -pinene (8.8%); and the *Piper hispidum* oil had β -caryophyllene (10.5%), α -humulene (9.5%), δ -3-carene (9.1%), α -copane (7.3%), limonene (6.9%), caryophyllene oxide (5.9%) and β -selinene (5.1%) (da Silva *et al.*, 2014).

Essential oils from the *Piperaceae* family are characterized by the presence of sesquiterpenes and derivatives of such oxygenates in their chemical composition; therefore, there are some similarities in the composition of these species (Chellappandian *et al.*, 2018). Monoterpenes and sesquiterpenes are a significant and very versatile class of secondary metabolites that are commercially used in food, cosmetic, pharmaceutical, biotechnology, and agricultural industries, among others (Koyama *et al.*, 2019).

Table 2 shows the results achieved for the repellent activity of the essential oil from *P. gorgonillense* on *T. castaneum*. The best repellent activity was at a concentration of 1%, with a repellency percentage of $75 \pm 10\%$ and $90 \pm 5\%$ at 2 and 4 h of exposure, respectively. This was compared to the commercial DEET repellent (N, N-diethyl-toluamide) at the same concentration with an activity of $76 \pm 6\%$ during the first 2 h and $78 \pm 5\%$ after 4 h (Tab. 2). These results show that the *P. gorgonillense* EO exhibited a higher repellent activity than the commercial repellent.

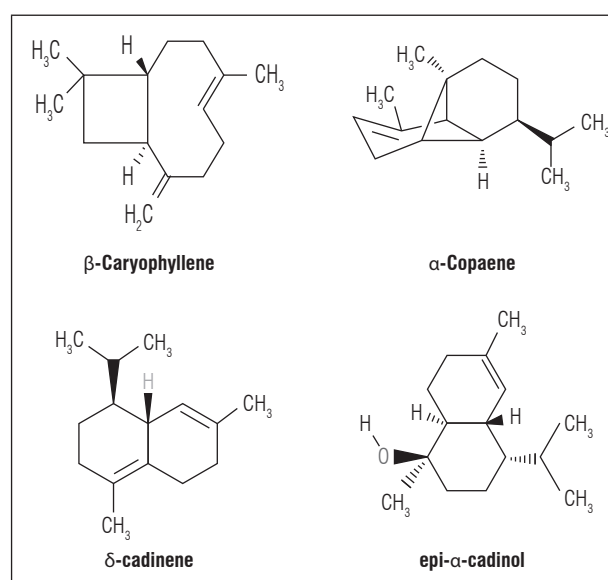


Figure 1. Chemical structure of the main compounds of the essential oil from *P. gorgonillense*. Source: MarvinSketch.

Table 2. Repellent activity of the essential oil from *P. gorgonillense* against *T. castaneum*.

Sample	Concentration (%)	Repellency (%)	
		Exposure time 2 h	Exposure time 4 h
<i>Piper gorgonillense</i> EO	0.0001	16 \pm 5	24 \pm 5
	0.001	32 \pm 8	36 \pm 5
	0.01	56 \pm 5	64 \pm 5
	0.1	68 \pm 4	74 \pm 5
	1.0	78 \pm 8	90 \pm 7
N,N-diethyl-toluamide (DEET)	0.0001	10 \pm 5	16 \pm 5
	0.001	16 \pm 5	18 \pm 8
	0.01	40 \pm 10	57 \pm 13
	0.1	50 \pm 5	59 \pm 11
	1.0	76 \pm 6	78 \pm 5

Repellent values = mean \pm SE of the four replicates (SE = standard error). Paired *t* test ($P < 0.05$).

After a statistical analysis of repellent activity results, bilateral significance was obtained with a value greater than 0.005, which were 0.106 at 2 h of exposure and 0.109 at 4 h of exposure; the intervals of confidence were $[(-2.55464) - (25.75464)]$ and $[(-2.78010) - (26.78010)]$ for 2 and 4 h of exposure, respectively. Lastly, the t-test showed an equal value for the two trials: the *P. gorgonillense* EO and DEET, with 1,648 at 2 h and 1,632 at 4 h for both. This analysis allowed us to infer that the *P. gorgonillense* EO and DEET had similar behaviors; therefore, it is possible to infer that the oil has a repellent activity very similar to that of a commercial repellent.

β -Caryophyllene has shown repellent activity against *Aedes aegypti* and *Anopheles minimus* (Nararak *et al.*, 2019), as well as against *Sitophilus granarius* (Plata *et al.*, 2018). In the study reported by Parisotto *et al.* (2020), β -caryophyllene acted as a powerful anti-inflammatory agent and demonstrated efficiency in the repair of skin tissue; β -caryophyllene provided anti-inflammatory and antiedemagnetic activity in *in vivo* tests of acute and chronic inflammation through a series of laboratory tests (De Oliveira-Tintino *et al.*, 2018). β -caryophyllene has shown an effective action against parasites such as *Trypanosoma cruzi* and *Leishmania brasiliensis*. Thanks to these results, this compound is considered an alternative against the aforementioned species (Leite *et al.*, 2013), i.e., δ -cadinene deployed larvicidal activity against the malaria vector *Anopheles stephensi*, (Govindarajan *et al.*, 2016). α -copaene has been evaluated as an insecticide against different types of insects, including the beetle, *Xyleborus glabratus* Eichhoff (Kendra *et al.*, 2016), the beetle *Euwallacea fornicatus* Eichhoff (Kendra *et al.*, 2017), the fly *Bactrocera oleae* (Rossi) (de Alfonso *et al.*, 2014), and *Ceratitis capitata* (Flat *et al.*, 1994).

Different δ -cadinene activities have also been reported, such as anticancer (Xu *et al.*, 2015), antifungal (Kundu *et al.*, 2013), and acaricidal activities (Guo *et al.*, 2017).

The fumigant activity results for the *P. gorgonillense* essential oil are shown in figure 2. The *P. gorgonillense* EO reached a fumigant activity of 100% at a concentration of $350 \mu\text{g mL}^{-1}$ air. Essential oils are considered fumigants because of their physical properties of low molecular weight, low boiling point and high volatility (Zhang *et al.*, 2017).

Table 3 presents the mean lethal concentrations (LC₅₀) obtained for the essential oil of *P. gorgonillense* in three exposure periods. The fumigant toxicity of the EO was evaluated on adult *T. castaneum* weevils. The results of the probit analysis showed that the pirimiphos methyl (positive control), at 24 h of exposure, obtained an LC₅₀ = $188,673 \mu\text{g mL}^{-1}$ air, LC₅₀ = $84,2145 \mu\text{g mL}^{-1}$ air at 48 h, and LC₅₀ = 68.0253 at 72 h, with pirimiphos-methyl being 2.3493 times more toxic in the first 24 h, 4.13622 times, more toxic in the first 48 h and 3.4261 times more lethal than the studied essential because the *P. gorgonillense* EO yielded an LC₅₀ = $443.262 \mu\text{g mL}^{-1}$ air at 24 h, an LC₅₀ = $348,328 \mu\text{g mL}^{-1}$ air at 48 h and an LC₅₀ = $233,066 \mu\text{g mL}^{-1}$ air at 72 h of exposure.

On the other hand, several studies have reported the repellent and/or insecticidal activity of essential oils from plants of the *Piper* genus on *Tribolium castaneum*, i.e. *Piper nigrum*, *Piper dilatatum*, *Piper aduncum*, *Piper divericatum* and *Piper* sp. (Scott *et al.*, 2008; Jaramillo-Colorado *et al.*, 2019a); *P. guineense* (Adarkwah *et al.*, 2018); and *Piper cubeba* (Chaubey, 2011); among others. The *P. gorgonillense* EO evaluated in this study

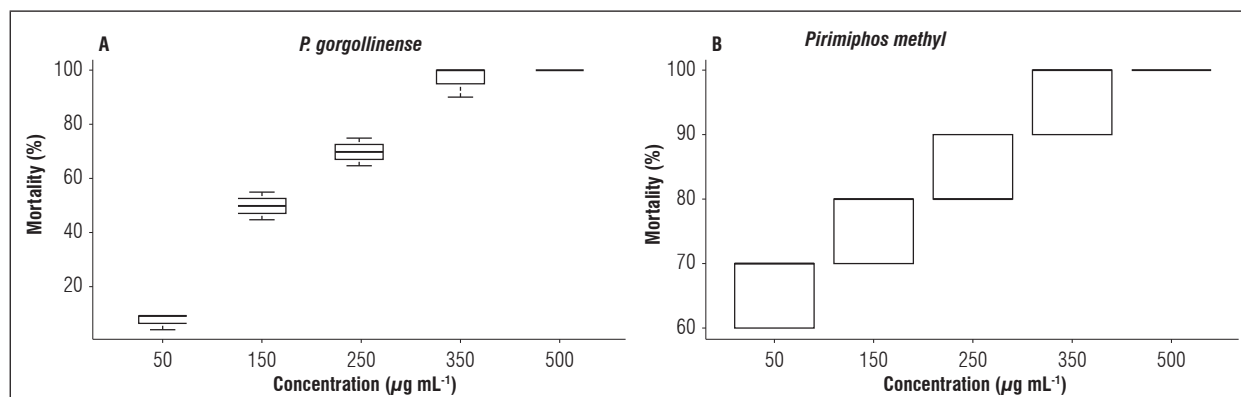


Figure 2. Fumigant activity of *P. gorgonillense* essential oil and pirimiphos methyl.

Table 3. Toxicity of the essential oil *P. gorgonillense* and pirimiphos- methyl against *T. castaneum*.

Treatments, <i>n</i> =5	Period (h)	LC ₅₀ (95% FL)	LC ₉₅ (95% FL)	X ² (df)	Slope ± SE
<i>P. gorgonillense</i>	24	443.262 [395.696; 495.943]	938.338 [831.046; 1100.81]	8.345 (3)	1.79 ± 0.18
	48	348.328 [315.464; 382.645]	671.424 [608.674; 760.233]	6.776 (3)	1.77 ± 0.17
	72	233.066 [202.345; 262.862]	515.145 [465.407; 583.398]	4.011 (2)	1.35 ± 0.16
Commercial insecticide (methyl pirimiphos)	24	188.673 [114.824; 246.333]	333.766 [277.547; 394.697]	1.449 (3)	0.0198 ± 0.0015
	48	84.2145 [77.023; 147.414]	216.133 [207.333; 320.308]	1.758 (3)	0.0159 ± 0.0015
	72	68.0253 [54.157; 79.856]	153.084 [143.502; 177.335]	0.193 (2)	0.0136 ± 0.0012

showed significant fumigant and repellent activity. According to the literature, the major sesquiterpenes found in the oil, such as β-caryophyllene, α-copaene and δ-cadinene, have exhibited insecticidal and/or repellent properties. Kim *et al.* (2010) reported that the fumigant and repellent activity of essential oils are related to the presence of monoterpenes, such as α-pinene, δ-cymene, carvacrol thymol, and α-and myrcene, and sesquiterpene compounds, such as caryophyllene oxide and caryophyllene (Kim *et al.*, 2010).

CONCLUSION

The results showed that the essential oil of *P. gorgonillense* and its terpenoid components deploy fumigant and repellent activity against *T. castaneum*, suggesting its potential for protecting stored grains.

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