Accepted manuscript

Elguedo-Vega L., B.E. Jaramillo-Colorado, and N. Pino-Benitez. 2024. Chemical composition and insecticidal activity of essential oils from *Piper coruscans*, *Piper ottoniaefolium* and *Piper reticulatum* against *Sitophilus zeamais* Motschulsky. Revista Colombiana de Ciencias Hortícolas 18(3), e18174. Doi: https://doi.org/10.17584/rcch.2024v18i3.18174

Chemical composition and insecticidal activity of essential oils from *Piper coruscans* Kunt, *Piper ottoniaefolium* C. DC. and *Piper reticulatum* L. against *Sitophilus zeamais* Motschulsky

Composición química y actividad insecticida de aceites esenciales de *Piper coruscans* Kunt, *Piper ottoniaefolium* C. DC. y *Piper reticulatum* L. contra *Sitophilus zeamais* Motschulsky

LENIMAR ELGUEDO-VEGA[1]

BEATRIZ EUGENIA JARAMILLO-COLORADO[1, 3]

NAYIBE PINO-BENÍTEZ[2]

[1] Universidad de Cartagena, Grupo de Investigaciones Agroquímicas, Cartagena de Indias (Colombia). ORCID Elguedo-Vega L.: https://orcid.org/0009-0002-0720-5909; ORCID Jaramillo-Colorado B.E.: https://orcid.org/0000-0003-1708-2367

[2] Universidad Tecnológica del Chocó, Grupo de Productos Naturales, Quibdo (Colombia). ORCID Pino-Benitez N.: https://orcid.org/0000-0001-7471-8697

[3] Corresponding author. bjaramilloc@unicartagena.edu.co

***Piper coruscans* Kunt (left), *Piper ottoniaefolium* C. DC. (center) and *Piper reticulatum* L. (right).** Photos: N. Pino-Benítez

Last name: ELGUEDO-VEGA / JARAMILLO-COLORADO / PINO-BENITEZ

Short title: CHEMICAL COMPOSITION AND INSECTICIDAL ACTIVITY OF *PIPER* SPECIES ESSENTIAL OILS AGAINST *SITOPHILUS ZEAMAIS*

Accepted manuscript

Elguedo-Vega L., B.E. Jaramillo-Colorado, and N. Pino-Benitez. 2024. Chemical composition and insecticidal activity of essential oils from *Piper coruscans*, *Piper ottoniaefolium* and *Piper reticulatum* against *Sitophilus zeamais* Motschulsky. Revista Colombiana de Ciencias Hortícolas 18(3), e18174. Doi: https://doi.org/10.17584/rcch.2024v18i3.18174

Doi: https://doi.org/10.17584/rcch.2024v18i3.18174

Received: 18-09-2024 Accepted: 16-10-2024 Published: 20-11-2024

ABSTRACT

Biocides based on essential oils (EOs) have emerged as a novel class of pest control agents due to their multifaceted bioactive properties and natural origin, which confers a reduced environmental impact. This research focused on the study of the volatile chemical composition and the repellent and fumigant activities of EOs extracted from the aerials parts of *Piper coruscans* Kunt (1815), *Piper ottoniaefolium* C. DC (1886) and *Piper reticulatum* L. (1753), originating from the Colombian Pacific flora, on the corn weevil *Sitophilus zeamais* Motschulsky (1855). The EOs were obtained by hydrodistillation and identified by GC–MS. The main components found in *P. coruscans* were caryophyllene oxide (31.75%), and β-selinene (10.29%), in *P. ottoniaefolium* were β-bisabolene (14.46%), and α-curcumene (8.36%), and in *P. reticulatum* were caryophyllene oxide (9.44%), and β-caryophyllene (9.01%). The repellent activity was determined by the preference area method, Area method, at 2.5 μg cm^{-2} and 2 h of exposure in which, the EO of *P. ottoniaefolium* obtained a percentage of repellency of 83.3%, higher than *P. coruscans* and *P. reticulatum* 66.7% and 36.7%, respectively against *S. zeamais*. Similarly, in the fumigant activity, the *P. ottoniaefolium* EO was more effective with an LC_{50} = 3.56 μL cm^{-3} compared to *P. reticulatum* = 6.12 μL cm^{-3} and *P. coruscans* = 7.43 μL cm^{-3}. The results indicated that the EOs of these three *Piperaceae*–have considerable insecticidal potential against *S. zeamais,* and they could be an alternative for the formulation of new biopesticide products.

Additional key words: Piperaceae; repellent; fumigant; biopesticide; terpens.

RESUMEN

Los biocidas basados en aceites esenciales (AE) han surgido como una nueva clase de agentes para el control de plagas debido a sus múltiples propiedades bioactivas y a su origen natural, que les confiere un reducido impacto ambiental. Esta investigación se centró en el estudio de la composición química volátil y las actividades repelente y fumigante de los AE extraídos de las partes aéreas de *Piper coruscans* Kunt (1815), *Piper ottoniaefolium* C. DC (1886) y *Piper reticulatum* L. (1753), originarios de la flora del Pacífico colombiano, sobre el gorgojo del maíz *Sitophilus zeamais* Motschulsky (1855). Los AE se obtuvieron por hidrodestilación y se identificaron por GC–MS. Los principales componentes encontrados en *P. coruscans* fueron óxido de cariofileno (31.75%) y β-selineno (10.29%), en *P. ottoniaefolium* fueron β-bisaboleno (14.46%) y α-curcumeno (8.36%), y en *P. reticulatum* fueron óxido de cariofileno (9.44%) y β-cariofileno (9.01%). La actividad repelente se determinó por el método del área de preferencia, a 2.5 µg cm^{-2} y 2 h de exposición, en la que el AE de *P. ottoniaefolium* obtuvo un porcentaje de repelencia del 83.3%, superior al de *P. coruscans* y *P. reticulatum*, 66.7% y 36.7%, respectivamente, frente a *S. zeamais*. Del mismo modo, en la actividad fumigante, el AE de *P. ottoniaefolium* fue más eficaz, con una CL_{50} = 3.56 µL cm^{-3}, en comparación con *P. reticulatum* = 6.12 µL cm^{-3} y *P. coruscans* = 7.43 µL cm^{-3}. Los resultados indicaron que los AE de estas tres *Piperaceae* tienen un considerable potencial insecticida contra *S. zeamais* y podrían ser una alternativa para la formulación de nuevos productos bioplaguicidas.

Palabras clave adicionales: Piperaceae; repelente; fumigante; bioplaguicida; terpenos.

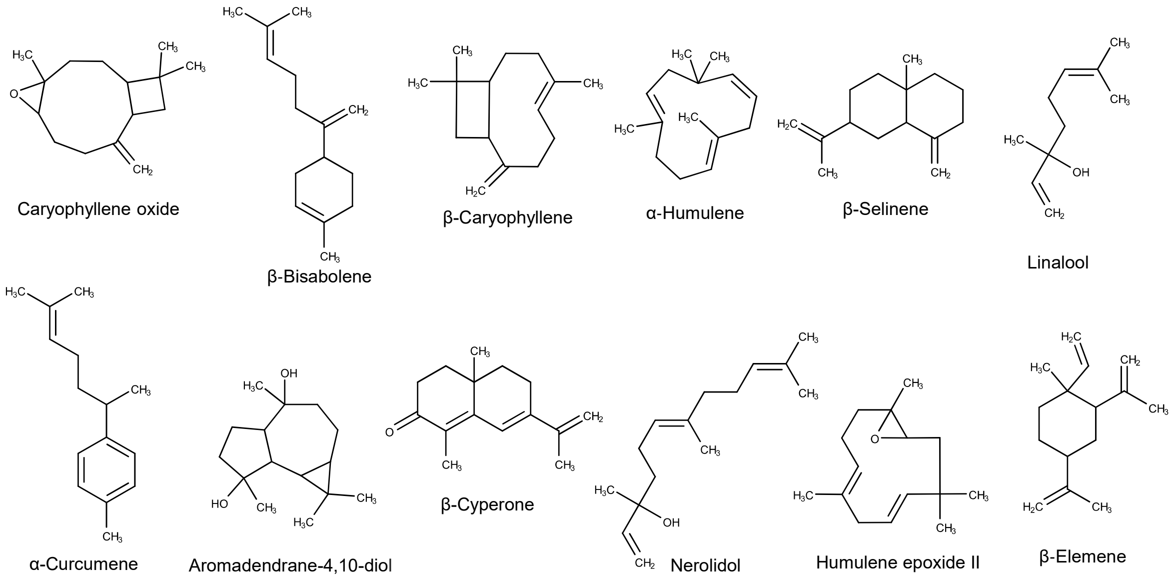
INTRODUCTION

Approximately 250 insect species attack crops, grains, and their products during storage. About twenty are of utmost importance (Manosathiyadevan *et al*., 2017). Among the most detrimental is the brown corn beetle *Sitophilus zeamais* Motschulsky, which receives its common name from the coloration and infestation habits in the grains of *Zea mays* L. This pest has its origin in Asia but is currently distributed throughout the world (Corrêa *et al*., 2017). Among the characteristics that define it as a pest of agricultural interest are its flight capacity, walking capacity, body mass, and grain consumption, in which the organisms feed on the embryo and thus reduce the germination percentage of the seeds (Choden *et al*., 2021). Larvae and adults cause the most significant damage to the grain. Especially the females, since they oviposit inside the seed and seal it with a gelatinous secretion, allowing the complete development of the new individual inside the grain. For this reason, most of the strategies to control *S. zeamais* are carried out on adults (Nwosu, 2018). Pesticides based on synthetic compounds (e.g., organophosphates, pyrethroids, carbamates, etc.) are used to control and minimize pests in crops and stored products (Hamel *et al*., 2020). These chemical substances are released into the environment to eradicate, prevent, control, repel, or mitigate pests and weeds in agricultural, domestic, and industrial environments (Islam *et al*., 2022). However, these compounds can generate contamination of soil, water, air, and food, affecting biodiversity and ecosystems due to their toxicological characteristics (Nwosu, 2018). Additionally, they can cause acute or chronic intoxication in humans and animals that in contact or consume them (Eijsackers and Maboeta, 2023). Some synthetic pesticides may be carcinogenic, mutagenic, teratogenic, or endocrine disruptors; this has led to their prohibition in some cases (Fernández *et al*., 2023).

Biopesticides appear as a more ecological and safer alternative for pest management (Bhavya *et al*., 2021; Chaudhari *et al*., 2021; Stejskal *et al*., 2021). Among these, we find those based on essential oils (EOs), which, in addition to presenting functional capacities like a synthetic pesticide, are biodegradable (Ram and Singh, 2021). EOs extracted from aromatic plants have long been of scientific relevance due to their biocidal properties against insects. They show insecticidal and fumigant actions against several pests of agronomic and medical importance, as well as against plant pathogens (Smith *et al*., 2018; Khursheed *et al*., 2022).

Around 6.500 plant species have been studied for their insecticidal properties. More than 2.500 belong to 235 families with biocidal activity (Giraldo-Rivera and Guerrero-Alvarez, 2019). Among the families with biopesticidal activity are the Piperaceae (Andrés *et al*., 2017; Jaramillo-Colorado *et al*., 2019; de Souza *et al*., 2020; Le *et al*., 2022). However, the insecticidal activity of the EOs of this family has been little evaluated in *S. zeamais* (Peschiutta *et al*., 2022). The Piperaceae family comprises approximately five genders and more than 3,000 species. The genus *Piper* L. is the largest of this family and the second largest in the world with 2.171 species (Amorim *et al*., 2023); cosmopolitan distribution, tropical and subtropical (Jaramillo and Manos, 2001). This genus has most of its diversity in humid forests, mountains, and lowlands. They are small trees, shrubs, ivies, and in smaller proportion hemiepiphytes. The leaves are simple, lanceolate, and alternate; the thick stems and the flowers are arranged in peduncular or erect inflorescences

(Jaramillo *et al.*, 2004). Approximately 1.900 taxa are distributed in tropical America (Amorim *et al.*, 2023), and only about 50 species have been studied for biocidal activity (Carmona-Hernández *et al.*, 2016). In terms of chemical components, the species of the *Piper* genus can present terpenes (piperitone, linalool, selinene), propenylphenols (apiol, safrole), amides (piperidine), among others (Whitehead *et al.*, 2013; Chandra *et al.*, 2014; Silva *et al.*, 2019). These components are potential biopesticides against pests. Some compounds obtained in this study are shown in figure 1.



Caryophyllene oxide

β-Bisabolene

β-Caryophyllene

α-Humulene

β-Selinene

Linalool

α-Curcumene

Aromadendrane-4,10-diol

β-Cyperone

Nerolidol

Humulene epoxide II

β-Elemene

Figure 1. Chemical structure of the main compounds found in the EOs of *P. coruscans, P. ottoniaefolium,* and *P. reticulatum*. Developed in: *MarvinSketch ver. 23.2.*

To date, for *P. ottoniaefolium, P. reticulatum* and *P. coruscans*, no biological activity against the *S. seamaiz*, has been reported, but biological activity against some fungi, insects and parasites has been reported (Santana *et al.*, 2016; Whitehead and Bowers, 2014; Ruiz-Vásquez *et al.*, 2022; Vásquez-Ocmín *et al.*, 2022). For these reasons, the present research seeks to determine the biocidal activity of the EOs to *P. ottoniaefolium*, *P. reticulatum* and *P. coruscans*, obtained from the Department of Chocó, Colombia and thus promote their potential use as insecticidal agents against *S. zeamais*.

MATERIALS AND METHODS

Plant material

Specimens of the three Piperaceae were collected in March 2022 from localities in the department of Chocó (Colombia). Specimens of *P. coruscans* were obtained from the municipality Garcia Gomez (4°10'0.012" N, 77°25'0.011" W), *P. ottoniaefolium* in the vicinity of the Universidad Tecnológica del Chocó (5°40'54" N, 5°3'49" W) and *P. reticulatum* in the municipality Lloro (5°36'4" N, 76°22'26" W). One specimen of each species was deposited in the National Herbarium of Colombia (COL) with voucher codes 552836 (*P. coruscans*), 522551 (*P. ottoniaefolium*), and 514401 (*P. reticulatum*).

Essential oils extraction

The essential oils were extracted by the hydrodistillation (HD) technique using a Clevenger-type apparatus, as described by Jaramillo *et al.* (2012). 500 g of finely chopped fresh leaves and distilled water (2-3 L) were added to a flask (5 L). The extraction time was 2-3 h. The EOs obtained were collected in vials (5 mL) and then anhydrous sodium sulfate (Na_2SO_4) was added to remove traces of water. Finally, the EOs were transferred to new vials, where the extraction yield was determined by the equation: $Yiel(\%) = M_{EO}/M_{PM} * 100\%$, where M_{EO} are the grams of essential oil obtained, and M_{PM} grams of plant material used in the extraction. They were then stored at 0-4°C, until chromatographic analysis, and bioassays were performed.

Gas chromatography analysis

Chromatographic analysis was performed on an Agilent 7890A series gas chromatograph coupled to an Agilent MSD 5975C mass spectrometry detector (GC-MS), using an HP-5MS capillary column (350°C, 30 m × 250 µm id × 0.25 µm pd), using the methodology proposed by Adams (2017), with some modifications. GC–MS was used under the following conditions: 1 µL of the diluted 1% v/v EOs in dichloromethane was injected in split-less mode (230°C). The initial oven temperature was 50°C for 2 min and a ramp of @3°C/min up to 250°C was programmed. The carrier gas used was He, with a pressure of 12.6 psi at a rate of 1 mL min^{-1}. Mass spectra were obtained by electron impact ionization at 70 eV energy, with a mass acquisition range of 30-700 m/z. The identification of the constituents of the chemical composition was performed by comparing the mass spectra obtained with the spectra available in the NIST08 library databases, and the Kovats retention indices were determined by comparing with the retention times obtained from an internal standard of *n*-alkanes (C_7-C_{40}), under the same analysis conditions.

Growth and identification of target insects

Adult specimens of *S. zeamais* were obtained from grain stored in a local market and were taxonomically identified, according to Rees (2007). Insect rearing was carried out at the Agrochemical Research Laboratory, University of Cartagena, in 4 L glass jars with a diet of 1.5 kg corn and 500 g barley. Conditions were maintained at 30±2°C temperature, 70% relative humidity, and 12:12 (D/N) photoperiods.

Bioassays insecticidal activity

Repellent activity

The EOs of *P. coruscans*, *P. ottoniaefolium, P. reticulatum*, and a commercial repellent, Stay Off® (3-(N-acetyl-N-butylamino) ethyl propionate, 15%) were evaluated on 7-10 d old, unsexed adult weevils. In addition, two compounds, caryophyllene oxide (Sigma-Aldrich, 95%) and linalool (Merck KGaA, 97%), were studied. This activity was based on the preference area method, according to Jaramillo-Colorado *et al*. (2014) with some modifications. In the interior of 9 cm Petri dishes, discs of Whatman® N°1 filter paper of 9 cm in diameter cut in half were placed, resulting in two working areas. To each half of the paper, 100 µL of different concentrations of the EOs, the standards, and the commercial product dissolved in acetone were added (0.31, 0.62, 1.25 y 2.50 µg cm^{-2}). The other half of the paper was used as a control, with the addition of acetone. The paper disks were dried in a fume hood for 10 min to evaporate the solvent. After this time, 20 adult specimens of *S. zeamais* were added to the center of the petri dishes. These were then covered and sealed with kerosene paper (Parafilm®) to prevent the escape of the insects. The assay was carried out in total darkness. Finally, after 2, 4, and 6 h of exposure time, the number of individuals present in the treated and untreated areas was counted. The percentage of repellency (%PR) was calculated by $\%PR = [(Nc - Nt) / (Nc + Nt) \times 100]$, where Nc is number of insects present in the control and Nt number of insects in the treated area. Each treatment was performed in triplicate and the experiment twice.

Fumigation activity

The toxic effect of *P. coruscans*, *P. ottoniaefolium*, *P. reticulatum*, and D-WT (Chlorpyrifos, 44%) were evaluated on a sample of 20 unsexed *S. zeamais* specimens, aged 7 to 10 d, following the methodology described by Brito *et al*. (2021). Additionally, two compounds were tested: caryophyllene oxide (Sigma-Aldrich, 95%) and linalool (Merck KGaA, 97%). Filter paper discs (Whatman® N° 1, 2.4 cm diameter) treated with 150 µL of the different concentrations of each treatment in acetone (2.5, 5.0, 10, and 20 $\mu L\ cm^{-3}$ air) were placed in 20 cm^3 screw cap vials. The filter paper discs were dried in an extraction hood for 15 min. Subsequently, they were introduced into the vials supporting them on the lids to avoid contact with the weevils. Acetone was used as a control. The test was carried out in complete darkness, and after 24 h, the number of dead was counted (those that did not show movement of legs or antennae). Each treatment was carried out in triplicate. With the results obtained, the percentage of mortality ($\%M$) was calculated and corrected with the equation of Abbott (1925). In which, $\%M = [(\%Mt - \%Mc) / (100 - \%Mc)]*100$, where is $\%Mt$ mortality with the treatment and $\%Mc$ mortality with the control. Finally, LC_{50} and LC_{95} were calculated for each treatment.

Statistical analysis

A multifactorial analysis of variance was performed to determine significant concentration ratios of concentration and time in the repellency bioassays, allowing to verify whether the percentage of repellency ($\%PR$) depended on the concentration or the exposure time. The repellent effect of the EO was compared with the commercial product by *t*-test ($P<0.05$). On the other hand, from the results obtained in the insecticidal activity, LC_{50} and LC_{95} were determined by linear regression of Probit analysis with parameters of

$P<0.05$, indicating a confidence level of 95%. In addition, the insecticidal effects of the EOs against the commercial product were compared by ANOVA performing a least squares mortality adjustment. Means were compared by a Tuckey test ($P<0.05$). All statistical analyses were performed using Statgraphics Centurion® v.19 software.

RESULTS AND DISCUSSION

Extraction yield

Of the three *Piper* species used in this study, the highest amount of essential oil was obtained from *P. ottoniaefolium*, while hydrodistillation of *P. coruscans* produced the lowest yield (Tab. 1). The plant material of *P. reticulatum* was richer in essential oil than *P. coruscans* and lower than *P. ottoniaefolium*; however, it recorded the highest density of the three oils (Tab. 1).

Table 1. Yields and characteristics of essential oil (EO) from *Piper coruscans, P. ottoniaefolium* and *P. reticulatum.*

EO	**Yield (%)**	**Density (g mL^{-1})**	**Color**
P. coruscans	0.32±0.01	0.86±0.03	Light yellow
P. ottoniaefolium	0.47±0.02	0.84±0.01	Light yellow
P. reticulatum	0.38±0.02	0.92±0.01	Dark yellow

Average±standard deviation.

Gas chromatography analysis

The main compounds of the EOs of the three Piperaceae are shown in table 2. For *P. coruscans*, 19 compounds were identified, being the major components the caryophyllene oxide (31.75%), *β*-selinene (10.29%), *β*-caryophyllene (7.12%), humulene epoxide II (6.07%), and *β*-elemene (5.57%). In *P. ottoniaefolium*, 45 compounds were identified. The principal compounds found were *β*-bisabolene (14.46%), *α*-curcumene (8.36%), 4,10-aromadendranediol (7.86%), aromadendrene (5.46%), and *α*-bisabolene epoxide (5.44%). Finally, in *P. reticulatum*, 63 compounds were identified, where caryophyllene oxide

(9.44%), *β*-caryophyllene (9.01%), *β*-selinene (5.15%), *α*-copaene (5.05%), and *β*-cyperone (4.69%) were the main ones.

The EOs of the three *Piper* rare often classified in five groups (Da Silva *et al*., 2017), predominantly monoterpenes (piperitone, *α*-pinene), sesquiterpenes (*β*-selinene, *β*-caryophyllene, *β*-elemene, aromadendrene, *β*-bisabolene, *α*-curcumene, *β*-cyperone, *α*-copaene), phenylpropanoids (safrol, apiole), sesquiterpene alcohols (4,10-aromadendranediol, isolongifololol), and oxygenated terpenoids (caryophyllene oxide, humulene epoxide II, *α*-bisabolene epoxide).

The EOs of three Piperaceae studied showed qualitative variations in contrast to oils from plants collected in other regions of tropical America. In the EO of *P. coruscans* collected in the Peruvian Amazon, the main components obtained were *β*-bisabolene (33.4%), and nerolidol (10.2%) (Ruiz-Vásquez *et al*., 2022). Gilardoni *et al*. (2020) reported for *P. coruscans* native from the coast and Amazon of Ecuador, (E)-*β*-caryophyllene (24.1%), *α*-humulene (11.6%), caryophyllene oxide (9.3%) and linalool (4.5%) as the major compounds in the leaves of this plant. The EO of *P. reticulatum* leaves collected in the Peruvian Amazon contained apiol (15.0%) and *D*-germacrene (12.6%). (Ruiz-Vásquez *et al*., 2022). In comparison, EO from a Brazilian region was characterized by *β*-elemene (24.6%) followed by *β*-caryophyllene (16.7%) (Luz *et al*., 2003) as principal compounds. This last compound coincides with the second principal compound reported in this research for *P. reticulatum*. Regarding the EO of *P. ottoniaefolium*, no reports of the volatile chemical composition of this oil were found. It was confirmed that, to date, this research is the first to describe the components of EOs. The main compound in this oil was *β*-bisabolene, a sesquiterpene found in the EO of various plants, including those of the genus *Piper*. These results coincide with the EO of *Piper lepturum* var. angustifolium, where *β*-bisabolene (17.72%) was the compound with the highest area, and in *Piper coruscans* was *β*-bisabolene, (33.4%) (Salehi *et al*., 2019; Ruiz-Vásquez *et al*., 2022).

The yield and chemical composition of essential oils can present chemical variations due to exogenous factors such as temperature, soil, light, rainfall, place of cultivation, and predators (Figueiredo *et al*., 2008; Karimi *et al*., 2020) and endogenous factors, such as volatile compound biosynthesis pathways and anatomical and physiological differences in plants (Barra, 2009).

Table 2. Volatile chemical composition of essential oils from *Piper coruscans* (Pco), *P. ottoniaefolium* (Pot) and *P. reticulatum* (Pre) by GC-MS.

Compounds	**Relative area (%)**			**IK**[a]
	Pco	**Pot**	**Pre**	
***α*-Pinene**	1.50	-	-	939
3-Carene	-	-	0.72	1,008
***p*-Cymene**	-	1.78	0.29	1,024
***β*-Terpinene**	1.65	-	-	1,054
Acetophenone	-	0.78	-	1,059
Linalool	3.29	0.84	2.49	1,096
1,3,8-*p*-Menthatriene	-	0.91	-	1,108
***trans*-Pinocarveol**	-	-	0.31	1,135
***D*-Limonene**	1.61	0.84	0.35	1,136
***trans*-Verbenol**	-	2.41	-	1,140
Menthone	-	0.79	0.60	1,148
***α*-Terpineol**	-	-	0.64	1,186
Myrtenol	-	-	0.35	1,194
Benzylacetone	-	0.79	-	1,228
Piperitone	1.43	-	2.48	1,249
Safrole	-	-	0.32	1,285
3-Undecanol	-	-	1.90	1,293
Panaxene	-	-	1.93	1,312
***α*-Cubebene**	-	0.94	3.28	1,351
***α*-Ylangene**	-	0.78	-	1,373
***α*-Copaene**	1.78	1.20	5.05	1,374
***β*-Cubebene**	-	0.81	-	1,387
***β*-Bourbonene**	-	1.08	0.35	1,388
Isolongifolene	2.39	-	0.55	1,389

***β*-Elemene**	5.57	0.83	3.11	1,390
***α*-Funebrene**	-	-	0.30	1,402
***β*-Caryophyllene**	7.12	2.25	9.01	1,419
***α*-Ionone**	-	-	0.38	1,428
***β*-Copaene**	-	-	0.38	1,430
***β*-Gurjunene**	-	-	0.31	1,431
***cis*-Thujopsene**	-	-	0.75	1,431
***trans*-*α*-Bergamotene**	-	0.80	0.32	1,432
***α*-Humullene**	3.28	1.03	-	1,438
***β*-Famesene**	-	1.58	-	1,443
***cis*-muurola-3,5-diene**	-	-	0.33	1,448
***α*-himachalene**	-	0.85	-	1,449
(*E*)-Cinnamic acid	3.04	-	-	1,452
***α*-Neoclovene**	-	1.72	-	1,452
Alloaromadendrene	-	-	0.32	1,461
9-*epi*-(*E*)-Caryophillene	-	-	1.75	1,466
***β*-Acoradiene**	-	-	0.29	1,469
***α*-Terpinyl isobutanoate**	-	-	0.48	1,471
***α*-Elemene**	-	-	3.33	1,477
***γ*-Muurolene**	-	2.25	0.60	1,479
Germacrene D	3.87	4.68	2.00	1,481
***β*-Selinene**	10.29	-	5.15	1,490
***epi*-Cubebol**	-	1.08	0.70	1494
Viridiflorene	-	-	0.78	1,496
***α*-Selinene**	-	1.02	-	1,498
***α*-Muurolene**	-	1.34	0.48	1,500
Cuparene	-	-	1.45	1,504
***β*-Bisabolene**	-	14.46	-	1,505
***γ*-Cadinene**	1.50	0.92	-	1,513
***α*-curcumene**		8.36	3.17	1,515
***β*-Sesquiphellandrene**	-	2.68	0.33	1,522

***trans*-Calamenene**	-	-	0.86	1,522
Nerolidol	2.88	1.20	0.48	1,532
***α*-Calacorene**	-	0.98	0.76	1,545
Elemol			0.60	1,549
Caryophyllene oxide	31.75	3.88	9.44	1583
Boronal	-	-	0.85	1,585
Salvial-4(14)-en-1-one	-	1.38	0.70	1,595
Widdrol	-	1.03	-	1,599
Ledol	-	-	0.38	1,602
Humulene epoxide II	6.07	-	3.23	1,608
Zingiberenol	-	1.13	-	1,626
Aromadendrane-4,10-diol	-	7.86	-	1,639
***τ*-Muurolol**	-	-	2.51	1,642
***β*-Eudesmol**	-	-	0.42	1,649
***τ*-Cadinol**	-	1.56	0.76	1,650
***α*-Cadinol**	-	1.59	-	1,654
Neointermedeol	4.63	-	-	1,660
Cadalene	-	-	0.82	1,675
***trans*-Sesquisabinene hydrate**	-	-	0.59	1,677
Apiole	-	-	1.11	1,678
Aromandendrene		5.46	0.43	1,680
Shyobunol	-	-	0.41	1,688
***cis*-Thujopsenal**	-	-	0.68	1,709
***α*-Cyperone**	4.57	-	-	1,727
Isolongifolol	-	3.07	3.99	1,729
***β*-Cyperone**	-	-	4.69	1,746
Cuparenal	-	1.36	-	1,753
***α*-Costol**	-	1.41	-	1,774
***α*-bisabolene epoxide**	-	5.44	3.36	1,814
Hexahydrofarnesyl acetone	-	1.69	-	1,834
Longifolenaldehyde	-	-	1.88	1,876

(*8S,14*)-Cedrandiol	-	-	0.57	1,889
Phytol	-	0.92	2.47	1,943
Oxygenated hydrocarbons	3.04	1.57	1.90	
Oxygenated monoterpenes	4.72	4.04	7.19	
Oxygenated sesquiterpenes	49.9	34.6	39.14	
Monoterpenes	4.76	3.53	1.74	
Sesquiterpenes	35.80	56.02	49.35	
Total	98.22	99.76	99.32	

^{a} Experimentally determined Kováts indices in a HP-5 column.

Repellent activity

The results of the repellent activity of the essential oils from the *Piper* species tested, and the commercial product, Stay Off® ((3-(N-acetyl-N-butylamino)), against *S. zeamais* are shown in table 3. The three essential oils showed the highest percentage of repellency at 2.5 µg cm^{-2} after 2, 4 and 6 h of exposure: *P. coruscans* (66.7±0.06%, 56.7±0.06%, 3.3±0.06%), *P. ottoniaefolium* (83.3±0.06%, 43.3±0.15%, -10.0±0.10%), and *P. reticulatum* (36.7±0.12%, 33.3±0.21%, -3.3±0.59%), respectively. Positive values indicate repellent activity, and negative values indicate attractive activity. For this reason, at 6 h of exposure, the EOs of *P. ottoniaefolium* and *P. reticulatum* showed attractive activity, and *P. coruscans* EO was the only one that showed repellent activity. The Stay Off® showed a highest percentage of repellency at 2, 4 and 6 h of exposure to 2.5 µg cm^{-2} (63.3±0.1%, 37.8±0.0%, -3.3±0.1%), respectively. Linalool showed a repellent effect at 2, 4, and 6 h of exposure 2.5 µg cm^{-2}, the highest percentage of repellency for this compound was obtained at 2, 4 and 6 h of exposure (60.0±0.00%, 53.3±0.12%, 20.0±0.10%) and 1.25 µg cm^{-2} (13.3±0.64%), respectively. Regarding caryophyllene oxide, it did not show repellent activity under any concentration at the exposure times tested.

The statistical analysis of the repellent activity, through the comparison of means by analysis of variance, for $P<0.05$, at 2 h of exposure, there were significant differences between *P. coruscans* (0.00002), *P. ottoniaefolium* (0.00048), *P. reticulatum* (0.00193), and linalool (0.00212), against the commercial product Stay Off®. In contrast, at 4 and 6 h of exposure no statistically significant differences for *P. coruscans* (0.10432, 0.732014), *P.*

ottoniaefolium (0.08093, 0.68728), *P. reticulatum* (0.11431, 0.33144), and linalool (0.10267, 0.20447), respectively.

Table 3. Repellent activity of essential oils from *Piper coruscans*, *P. ottoniaefolium* and *P. reticulatum*, linalool, caryophyllene oxide and Stay off® (commercial control) against *Sitophilus zeamais*.

Compounds	Concentration ($\mu g\ cm^{-2}$)	Percentage of repellency, according to exposure time (hours)		
		2	4	6
P. coruscans	2.50	66.7±0.06 a	56.7±0.06	3.3±0.25
	1.25	36.7±0.06 a	13.3±0.12	-16.7±0.15
	0.62	16.7±0.06 a	3.3±0.12	-36.7±0.12
	0.31	3.3±0.06 a	-6.7±0.12	-43.3±0.32
P. ottoniaefolium	2.50	83.3±0.06	43.3±0.06	-10.0±0.10
	1.25	46.7±0.06 a	33.3±0.12	-20.0±0.20
	0.62	43.3±0.15 a	0.0±0.10	-26.7±0.12
	0.31	13.3±0.12 a	-6.7±0.15	-33.3±0.15
P. reticulatum	2.50	36.7±0.12 a	33.3±0.21	-3.3±0.59
	1.25	26.7±0.12 a	16.7±0.45	-26.7±0.38
	0.62	13.3±0.06 a	3.3±0.21	-36.7±0.38
	0.31	10.0±0.10 a	-13.3±0.31	-50.0±0.10
Linalool	2.50	60.0±0.00 a	53.3±0.12	20.0±0.10
	1.25	30.0±0.10 a	23.3±0.55	13.3±0.64
	0.62	33.3±0.15 a	6.7±0.06	-10.0±0.10
	0.31	20.0±0.00 a	-6.7±0.12	-23,3±0.21
Caryophyllene oxide	2.50	-3.5±0.18	-18.3±0.14	-21.3±0.10
	1.25	-8.7±0.13	-24.1±0.03	-30.7±0.32
	0.62	-14.3±0.21	-37.2±0.12	-49.8±0.28
	0.31	-28.6±0.16	-44.2±0.31	-59.1±0.26
Stay off	2.50	63.3±0.1	37.8±0.0	-3.3±0.1
	1.25	30.0±0.1	5.5±0.1	-23.3±0.2
	0.62	3.3±0.1	-13.3±0.1	-20.0±0.1

	0.31	-16.7±0.2	-46.7±0.2	-11.6±0.2

[a] There is a statistically significant difference between the essential oil and the commercial repellent (P<0.05). Mean±standard deviation.

The confidence intervals at 2, 4 and 6 h of exposure for *P. coruscans* were:[0.32503; 0.74163], [-0.04667; 0.46334], [-0.12448; 0.17448]; for *P. ottoniaefolium*: [0.19697; 0.60302], [-0.02896; 0. 46229], [-0.15210; 0.10210]; for *P. reticulatum*: [0.16430; 0.63569], [-0.05659; 0.48992], [-0.10882; 0.30882] and linalool: [0.1684; 0.66493], [-0.05083; 0.51750], [-0.08300; 0.36633], respectively. Since, at 2 h of exposure in all treatments, the intervals do not contain the value 0, there are statistically significant differences between the means of the treatments with Stay Off® with a confidence level of 95%, contrary to the case seen at 4 and 6 h of exposure, where the value 0 is included in the confidence intervals. From the above, it is assumed that there are no significant differences between the means compared. Considering these results, the EOs of *P. coruscans*, *P. ottoniaefolium*, *P. reticulatum* and linalool did not show similarity with Stay off® at 2 h. From this, it can be indicated that the EOs have a repellent activity superior to the commercial repellent on *S. zeamais*.

The results obtained in this research show a high repellent activity of *Piper* EO against *S. zeamais*. This is consistent with published studies on the repellent effects of EOs from Piperaceae against several pests (Araújo *et al*., 2012; Vasantha-Srinivasan *et al*., 2018; Muñoz-Acevedo *et al*., 2023). Of the three tested EOs, *P. ottoniaefolium* showed a higher repellency percentage (83.3±0.06%) than *P. coruscans* (66.7±0.06%) and *P. reticulatum* (36.7±0.12%), at 2.5 µg cm^{-2}, after 2 h of exposure, on *S. zeamais*. This could be explained by the chemical composition of *P. ottoniaefolium* EO, of which β-bisabolene (a sesquiterpene) was identified as the primary compound. Previous studies have reported repellent potential in sesquiterpenes (Fraga *et al*., 2021; Jiménez-Durán *et al*., 2021). On the other hand, Wei *et al*. (2018) showed a high repellent effect of β-bisabolene against the cigarette beetle, *Lasioderma serricorne* Fabricius, and the booklouse *Liposcelis bostrychophila* Badonnel. However, after 4 and 6 h of exposure *P. coruscans* had the highest stability in repellent activity, being the only EO to show repellency at 2.5 µg cm^{-2} and 6 h (3.3±0.25%) in contrast to the EOs of *P. ottoniaefolium* (-20.0±0.20%) and *P.*

reticulatum (-3.3±0.59%) which resulted with attractive activity at all concentrations evaluated at the latter exposure time. This is one of the reasons why the effects are so differentiated, as the exposure time elapses (Chan *et al*., 2016; Kaur *et al*., 2021; Zhang *et al*., 2023). It is not possible to affirm that the repellent activity of EOs against insects is limited only to the potential of some of their main constituents since this effect could also be due to certain minority compounds or the antagonistic or synergistic behavior of several compounds (Dhifi *et al*., 2016; Dassanayake *et al*., 2021; Kaur *et al*., 2021).

The linalool standard (terpenoid alcohol) showed the highest percentage of repellency at 2.5 μg cm^{-2} after 2 h of exposure (60.0±0.00%). These results agree with those reported by Karemu *et al*. (2013), where this compound obtained a repellency of 63.3% at the highest dose tested (2.0 μg μL^{-1}). This compound has also been evaluated on several species of mosquitoes, resulting in high repellency rates (Müller *et al*., 2009; Dekker *et al*., 2011; Murtaza *et al*., 2023) and in beetles, lice, and flies (Yang *et al*., 2014; Wang *et al*., 2019; Papanastasiou *et al*., 2020).

Fumigant activity

The results of the percentage of mortality of the EOs of *P. coruscans, P. ottoniaefolium,* and *P. reticulatum* are shown in figure 2. The oils of *P. coruscans*, and *P. ottoniaefolium* reached mortality of 100% in 20 μL cm^{-3} air. In comparison, the oil of *P. reticulatum* at the same concentration showed a mortality of 73.3±0.31%. The tested substances: linalool, caryophyllene oxide, and the commercial insecticide D-WT (chlorpyrifos) showed mortalities of 96.7±2.87, 13.4±3.77, and 98.3±2.88%, in 20 μL cm^{-3}, respectively. At the lowest concentration tested, 2.5 μL cm^{-3} air, the behavior of the insecticidal activity of the EOs was *P. coruscans*, 15.0±0.05%, *P. ottoniaefolium*, 23.9±0.10%, and *P. reticulatum*, 23.8±0.08%. Similarly, linalool, caryophyllene oxide, and commercial insecticide reached mortalities of 50.1±5.03, 0, and 86.7±7.63%, respectively. Based on the above and considering that the mortality of the standards is higher than that of the EOs studied, they are considered fumigants against *S. zeamais*, since they must be applied in low doses to show their biocidal capacity, due to the physicochemical properties of these compounds (Wang *et al*., 2021).

The toxicity of EOs against insects is due to the rapid action of the principal metabolites present in their composition (Angane *et al*., 2022). This toxicity is generated by four routes: inhalation, contact, absorption, and ingestion. Similarly, several mechanisms of toxic action of EO against insects have been described, such as inhibition of acetylcholinesterase, blocking of gamma-aminobutyric acid (GABA) channels, interference with the neuromodulator octopamine, among others (Koyama and Heinbockel, 2020; Hung *et al*., 2022; Mattar *et al*., 2022; Mssillou *et al*., 2022). However, it has been confirmed that the minority components in EOs can also affect these mechanisms because they exert synergistic actions with the other compounds present in EOs (Yuan *et al*., 2019; Kim *et al*., 2021).

Table 4 shows the LC_{50} and LC_{95} against *S. zeamais* the EOs *P. coruscans*, *P. ottoniaefolium,* and *P. reticulatum*, the substances linalool, caryophyllene oxide and the commercial insecticide D-WT (Chlorpyrifos) after 24 h of exposure. The results of the *Probit* analysis showed that the EOs obtained LC_{50}, in µL cm^{-3} air, of 7.43 *(P. coruscans*), 3.56 (*P. ottoniaefolium*) and 6.12 (*P. reticulatum*), and LC_{95}, in µL cm^{-3} air, of 16.07, 5.95 and 28.75, respectively. When the analysis of variance was performed for the comparison of means by Tukey's test, with an $\alpha<0.05$, statistically significant differences were obtained between the insecticidal capacities of the EOs and linalool against the commercial insecticide DW-T. The p values obtained from the comparisons were *P. coruscans* (0.0002), *P. ottoniaefolium* (0.0060), *P. reticulatum* (0.0012) and linalool (0.0361*)*. The 95.0% confidence intervals for the mean difference were *P. coruscans* [0.4171, 0.6296], *P. ottoniaefolium* [0.5413, 0.7537], *P. reticulatum* [0.4637, 0.6762] and linalool [0.6729, 0. 8854]. Since the intervals do not contain the value 0, the existence of a statistically significant difference between the means of mortality of EOs and linalool against DW-T is reaffirmed. This allows estimating that the insecticidal capacity of the oils and linalool are not comparable to the commercial product.

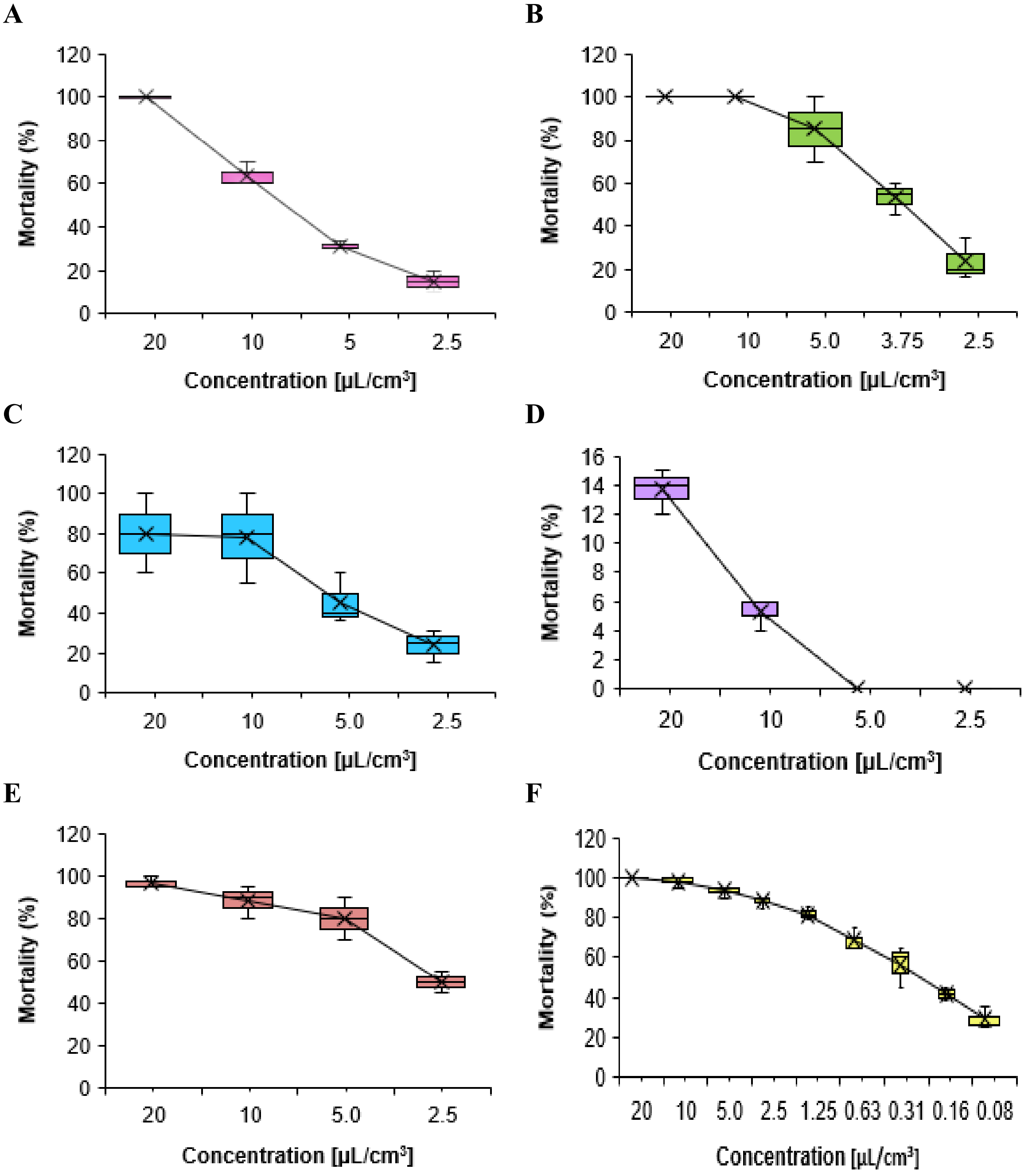


Figure 2. Mortality rate of *Sitophilus zeamais* at different concentrations of the essential oils *Piper coruscans* (A), *P. ottoniaefolium* (B) and *P. reticulatum* (C), the standards caryophyllene oxide (D) and linalool (E), and the commercial product D-WT chlorpyrifos (F) after 24 h of exposure.

Table 4. Toxicity of the essential oils *Piper coruscans, P. ottoniaefolium* and *P. reticulatum*, the commercial substances linalool, caryophyllene oxide and D-WT against *Sitophilus zeamais*.

Treatment	**LC_{50} (µL cm^{-3})[a]**	**LC_{95} (µL cm^{-3})[a]**	**X^{2b} (df)**	**Slop±sd**
Piper coruscans	7.43 [6.37; 8.59]	16.07 [13.98; 19.43]	25.72 (3)[c]	0.19±0.03
Piper ottoniaefolium	3.56 [3.22; 3.85]	5.95 [5.36; 6.99]	48.07 (4)	0.68±0.10
Piper reticulatum	6.12 [3.45; 8.81]	28.75 [21.61; 44.35]	27.98 (3)	0.16±0.03
Linalol	2.07 [1.20; 4.03]	17.45 [14.25; 23.75]	35.67 (3)	0.18±0.02
Caryophyllene oxide	-	-	-	-
D-WT (chlorpyrifos)	0.12 [0.11; 0.35]	5.57 [3.20; 7.25]	5.89 (4)	0.14±0.05

[a] LC_{50} and LC_{95}: insecticide concentrations required to kill 50 or 95% of *S. zeamais* adults, respectively (CI confidence interval at 95% error probability) (*n*=5).
[b] χ^2 Pearson's chi-square value.
[c] Degrees of freedom (df).

However, it should be considered that due to the toxicology of the commercial product, it is highly more harmful to health than EOs in any case. In addition, it should be taken into account that the insecticidal action can be affected by the volatility of the components of the product (Barra, 2009). Finally, caryophyllene oxide reached a mortality of 13.4±3.77% at 20 µL cm^{-3} air; these data are not sufficient to be able to estimate mean mortality values, therefore, they were not compared with the commercial product. To date, no insecticidal activity on *S. zeamais* has been described for the EOs of the Piperaceae included in this research.

Although, in several studies, the biopesticidal action of *Piper* spp. EOs on *S. zeamais* has been demonstrated. Whitehead and Bowers (2014) tested amide extracts obtained from *P. reticulatum* on *Sibaria englemani*, an antagonistic fruit pest. Vásquez-Ocmín *et al.* (2022) reported antiplasmodial activity of ethanolic extracts of *P. coruscans* on *Plasmodium falciparum*. This is the first report of biocidal activity of *P. ottoniaefolium* EO.

The results obtained in the present study reveal insecticidal activity by fumigant action of EOs of *P. coruscans*, *P. ottoniaefolium* and *P. reticulatum* against *S. zeamais*. The effectiveness of the EOs may be due to the compounds present in the EOs, mainly monoterpenes (Yildirim *et al*., 2013). Such is the case of linalool, the substance assayed in this study, which showed 98% mortality at the highest dose at 24 h of exposure. These results coincide with those reported by Kamanula *et al*. (2017), where, linalool obtained 100% mortality against *S. zeamais* at 48 h of exposure. It should be noted that the commercial insecticide D-WT (chlorpyrifos) was tested up to a concentration of 0.08 µL cm^{-3}, to obtain a mortality of 42.2±0.07% because it was highly insecticidal (Fig. 2). Caryophyllene oxide reported low mortality at all doses analyzed on

S. zeamais; this is in agreement with the reports by (Ma *et al*., 2020), where this oxygenated sesquiterpene oxide showed low fumigant toxicity against *S. zeamais* and *M. japonica*. However, the antifungal activity of caryophyllene oxide against several species of fungi has been demonstrated (Jassal *et al*., 2021), as an antiproliferative agent in the treatment of PC-3 prostate cancer cells (Delgado *et al*., 2021). In addition, it has shown important anticholinesterase and antioxidant capacities (Karakaya *et al*., 2020).

CONCLUSIONS

In the present investigation, the chemical composition and insecticidal activity of the EOs of three Piperaceae were studied, showing their potential as effective alternatives for the control of *S. zeamais*. The results of the chromatographic analysis indicated that the majority compounds found in *Piper coruscans* were caryophyllene oxide (31.75%), and β-selinene (10.29%); in *P. ottoniaefolium* were the β-bisabolene (14.46%), and α-curcumene (8.36%), and in *P. reticulatum* were caryophyllene oxide (9.44%), and β-caryophyllene (9.01%). The volatile chemical composition of *P. ottoniaefolium* EO was described for the first time. The EO of *P. ottoniaefolium* showed a higher repellency percentage than the other two oils (83.3%) at 2 hours (h) of exposure and 2.5 µg cm^{-2}. In addition, the *P. coruscans* EO showed the most stable repellency percentage during the test, 2 h (66.7±0.06), 4 h (56.7±0.06) and 6 h (3.3±0.25) at 2.5 µg cm^{-2}. The LC_{50} of the EOs of *P. coruscans*, *P. ottoniaefolium* and *P. reticulatum* were (7.43, 5.56 and 6.12 in µL cm^{-3}, respectively) and of the linalool standard 2.07 µL cm^{-3}. On the other hand, from the comparison of means between the EOs with the commercial insecticide product (DW-T), statistically significant differences were obtained, due to their high toxicity. However, the results of the LC_{50} and LC_{95} showed these essential oils as potential botanical insecticides.

Elguedo-Vega L., B.E. Jaramillo-Colorado, and N. Pino-Benitez. 2024. Chemical composition and insecticidal activity of essential oils from *Piper coruscans*, *Piper ottoniaefolium* and *Piper reticulatum* against *Sitophilus zeamais* Motschulsky. Revista Colombiana de Ciencias Hortícolas 18(3), e18174. Doi: https://doi.org/10.17584/rcch.2024v18i3.18174

Therefore, the essential oils of *P. coruscans*, *P. ottoniaefolium*, and *P. reticulatum* can be a considerable alternative in controlling *S. zeamais* and creating new biopesticides.

Conflict of interest: the manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

BIBLIOGRAPHY REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18(2), 265-267. Doi: https://doi.org/10.1093/jee/18.2.265a

Adams, R.P. 2017. Identification of essential oil components by gas chromatography/mass spectroscopy. 4th ed. Allured Publishing Corporation, Carol Stream, IL.

Amorim, P.R.F., K.S.G. Kubota, and M. Carvalho-Silva. 2023. Trends and gaps in the knowledge of diversity, distribution and conservation of neotropical species of *Piper* (Piperaceae): a scoping review. Biodivers. Conserv. 32, 851-866. Doi: https://doi.org/10.1007/s10531-023-02551-9

Andrés, M.F., G.E. Rossa, E. Cassel, R.M.F. Vargas, O. Santana, C.E. Díaz, and A. González-Coloma. 2017. Biocidal effects of *Piper hispidinervum* (Piperaceae) essential oil and synergism among its main components. Food Chem. Toxicol. 109(Part 2), 1086-1092. Doi: https://doi.org/10.1016/j.fct.2017.04.017

Angane, M., S. Swift, K. Huang, C.A. Butts, and S.Y. Quek. 2022. Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. Foods 11(3), 464. Doi: https://doi.org/10.3390/foods11030464

Araújo, M.J.C., C.A.G. Câmara, F.S. Born, M.M. Moraes, and C.A. Badji. 2012. Acaricidal activity and repellency of essential oil from *Piper aduncum* and its components against *Tetranychus urticae*. Exp. Appl. Acarol. 57, 139-155. Doi: https://doi.org/10.1007/s10493-012-9545-x

Barra, A. 2009. Factors affecting chemical variability of essential oils: a review of recent developments. Nat. Prod. Commun. 4, 8. Doi: https://doi.org/10.1177/1934578X0900400827

Bhavya, M.L., S. Obulaxmi, and S.S. Devi. 2021. Efficacy of *Ocimum tenuiflorum* essential oil as grain protectant against coleopteran beetle, infesting stored pulses. J. Food Sci. Technol. 58, 1611-1616. Doi: https://doi.org/10.1007/s13197-020-04871-y

Brito, V.D., F. Achimón, R.P. Pizzolitto, A. Ramírez, E.A. Gómez, J.A. Zygadlo, and M.P. Zunino. 2021. An alternative to reduce the use of the synthetic insecticide against the maize weevil *Sitophilus zeamais* through the synergistic action of *Pimenta racemosa* and *Citrus sinensis* essential oils with chlorpyrifos. J. Pest Sci. 94, 409-421. Doi: https://doi.org/10.1007/s10340-020-01264-0

Carmona-Hernández, C., J.A. Lozada-García, M.J. Martínez-Hernández, and V.R. Torres-Pelayo. 2016. *Piper* L. genus potential as natural biocide. Wulfenia J. 23(6), 65-95.

Chan, W.-K., L.T.-H. Tan, K.-G. Chan, L.-H. Lee, and B.-H. Goh. 2016. Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. Molecules 21(5), 529. Doi: https://doi.org/10.3390/molecules21050529

Chandra, P., V. Bajpai, M. Srivastva, K.B.R. Kumar, and B. Kumar. 2014. Metabolic profiling of *Piper* species by direct analysis using real time mass spectrometry combined with principal component analysis. Anal. Methods 6(12), 4234-4239. Doi: https://doi.org/10.1039/C4AY00246F

Chaudhari, A.K., V.K. Singh, A. Kedia, S. Das, and N.K. Dubey. 2021. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. Environ. Sci. Pollut. Res. 28, 18918-18940. Doi: https://doi.org/10.1007/s11356-021-12841-w

Choden, S., U. Yangchen, and J. Tenzin. 2021. Evaluation on efficacy of *Piper nigrum* as a bio-pesticide against *Sitophilus zeamais*. Naresuan Univ. J. Sci. Technol. NUJST 29(2), 84-95. Doi: https://doi.org/10.14456/nujst.2021.19

Corrêa, A.S., C.C. Vinson, L.S. Braga, R.N.C. Guedes, and L.O. Oliveira. 2017. Ancient origin and recent range expansion of the maize weevil *Sitophilus zeamais*, and its genealogical relationship to the rice weevil *S. oryzae*. Bull. Entomol. Res. 107(1), 9-20. Doi: https://doi.org/10.1017/S0007485316000687

Da Silva, J.K., R. Da Trindade, N.S. Alves, P.L. Figueiredo, J.G.S. Maia, and W.N. Setzer. 2017. Essential oils from neotropical *Piper* species and their biological activities. Int. J. Mol. Sci. 18(12), 2571. Doi: https://doi.org/10.3390/ijms18122571

Dassanayake, M.K., C.H. Chong, T.-J. Khoo, A. Figiel, A. Szumny, and C.M. Choo. 2021. Synergistic field crop pest management properties of plant-derived essential oils in combination with synthetic pesticides and bioactive molecules: a review. Foods 10(9), 2016. Doi: https://doi.org/10.3390/foods10092016

de Souza, M.T., M.T. Souza, D. Bernardi, D. Krinski, D.J. Melo, D.C. Oliveira, M. Rakes, P.H.G. Zarbin, B.H.L.N.S. Maia, and M.A.C. Zawadneak. 2020. Chemical composition of essential oils of selected species of *Piper* and their insecticidal activity against *Drosophila suzukii* and *Trichopria anastrephae*. Environ. Sci. Pollut. Res. 27, 13056-13065. Doi: https://doi.org/10.1007/s11356-020-07871-9

Dekker, T., R. Ignell, M. Ghebru, R. Glinwood, and R. Hopkins. 2011. Identification of mosquito repellent odours from *Ocimum forskolei*. Parasit. Vectors 4, 183. Doi: https://doi.org/10.1186/1756-3305-4-183

Delgado, C., G. Mendez-Callejas, and C. Celis. 2021. Caryophyllene oxide, the active compound isolated from leaves of *Hymenaea courbaril* L. (Fabaceae) with antiproliferative and apoptotic effects on PC-3 androgen-independent prostate cancer cell line. Molecules 26(20), 6142. Doi: https://doi.org/10.3390/molecules26206142

Dhifi, W., S. Bellili, S. Jazi, N. Bahloul, and W. Mnif. 2016. Essential oils' chemical characterization and investigation of some biological activities: a critical review. Medicines 3(4), 25. Doi: https://doi.org/10.3390/medicines3040025

Eijsackers, H. and M. Maboeta. 2023. Pesticide impacts on soil life in southern Africa: consequences for soil quality and food security. Environ. Adv. 13, 100397. Doi: https://doi.org/10.1016/j.envadv.2023.100397

Fernández, M.R.F., S.M.L. Lavarías, V. Aparicio, K.E. Larsen, J.E. Colman, and A. Cortelezzi. 2023. Ecological risk assessment of pesticides in sediments of Pampean streams, Argentina. Chemosphere 313, 137598. Doi: https://doi.org/10.1016/j.chemosphere.2022.137598

Figueiredo, A.C., J.G. Barroso, L.G. Pedro, and J.J.C. Scheffer. 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr. J. 23(4), 213-226. Doi: https://doi.org/10.1002/ffj.1875

Fraga, B.M., C.E. Díaz, M. Bailén, and A. González-Coloma. 2021. Sesquiterpene lactones from *Artemisia absinthium*. Biotransformation and rearrangement of the insect antifeedant 3α-hydroxypelenolide. Plants 10(5), 891. Doi: https://doi.org/10.3390/plants10050891

Gilardoni, G., Y. Matute, and J. Ramírez. 2020. Chemical and enantioselective analysis of the leaf essential oil from *Piper coruscans* Kunth (Piperaceae), a costal and Amazonian native species of Ecuador. Plants 9(6), 791. Doi: https://doi.org/10.3390/plants9060791

Giraldo-Rivera, A.-I., G.-E. Guerrero-Alvarez. 2019. Botanical biopesticides: research and development trends, a focus on the Annonaceae family. Rev. Colomb. Cienc. Hortícolas 13(3), 371-383. Doi: https://doi.org/10.17584/rcch.2019vl3i3.9489

Hamel, D., V. Rozman, and A. Liška. 2020. Storage of cereals in warehouses with or without pesticides. Insects 11(12), 846. Doi: https://doi.org/10.3390/insects11120846

Hung, N.H., P.M. Quan, P. Satyal, D.N. Dai, V.V. Hoa, N.G. Huy, L.D. Giang, N.T. Ha, L.T. Huong, V.T. Hien, and W.N. Setzer. 2022. Acetylcholinesterase inhibitory activities of essential oils from Vietnamese traditional medicinal plants. Molecules 27(20), 7092. Doi: https://doi.org/10.3390/molecules27207092

Islam, M.A., S.M.N. Amin, M.A. Rahman, A.S. Juraimi, M.K. Uddin, C.L. Brown, and A. Arshad. 2022. Chronic effects of organic pesticides on the aquatic environment and human health: a review. Environ. Nanotechnol. Monit. Manag. 18, 100740. Doi: https://doi.org/10.1016/j.enmm.2022.100740

Jaramillo, M.A. and P.S. Manos. 2001. Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). Am. J. Bot. 88(4), 706-716. Doi: https://doi.org/10.2307/2657072

Jaramillo, M.A., P.S. Manos, and E.A. Zimmer. 2004. Phylogenetic relationships of the Perianthless Piperales: reconstructing the evolution of floral development. Int. J. Plant Sci. 165(3), 403-416. Doi: https://doi.org/10.1086/382803

Jaramillo, B.E., I.P. Martelo, and E. Duarte. 2012. Antioxidant and repellent activities of the essential oil from Colombian *Triphasia trifolia* (Burm. f.) P. Wilson. J. Agric. Food Chem. 60(25), 6364-6368. Doi: https://doi.org/10.1021/jf300461k

Jaramillo-Colorado, B.E., M.C. Karen, D.R. Edisson, S. Elena, and O.V. Jesus. 2014. Volatile secondary metabolites from Colombian *Croton malambo* (Karst) by different extraction methods and repellent activity of its essential oil. J. Essent. Oil Bear. Plants 17(5), 992-1001. Doi: https://doi.org/10.1080/0972060X.2014.895185

Jaramillo-Colorado, B.E., N. Pino-Benitez, and A. González-Coloma. 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. Doi: https://doi.org/10.1016/j.indcrop.2019.06.026

Jassal, K., S. Kaushal, R. Rashmi, and R. Rani. 2021. Antifungal potential of guava (*Psidium guajava*) leaves essential oil, major compounds: beta-caryophyllene and caryophyllene oxide. Arch. Phytopathol. Plant Prot. 54(19-20), 2034-2050. Doi: https://doi.org/10.1080/03235408.2021.1968287

Jiménez-Durán, A., J. Barrera-Cortés, L.P. Lina-García, R. Santillan, R.M. Soto-Hernández, A.C. Ramos-Valdivia, T. Ponce-Noyola, and E. Ríos-Leal. 2021. Biological activity of phytochemicals from agricultural wastes and weeds on *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Sustainability 13(24), 13896. Doi: https://doi.org/10.3390/su132413896

Kamanula, J.F., S.R. Belmain, D.R. Hall, D.I. Farman, D.J. Goyder, B.M. Mvumi, F.F. Masumbu, and P.C. Stevenson. 2017. Chemical variation and insecticidal activity of *Lippia javanica* (Burm. f.) Spreng essential oil against *Sitophilus zeamais* Motschulsky. Ind. Crops Prod. 110, 75-82. Doi: https://doi.org/10.1016/j.indcrop.2017.06.036

Elguedo-Vega L., B.E. Jaramillo-Colorado, and N. Pino-Benitez. 2024. Chemical composition and insecticidal activity of essential oils from *Piper coruscans*, *Piper ottoniaefolium* and *Piper reticulatum* against *Sitophilus zeamais* Motschulsky. Revista Colombiana de Ciencias Hortícolas 18(3), e18174. Doi: https://doi.org/10.17584/rcch.2024v18i3.18174

Karakaya, S., S.V. Yilmaz, Ö. Özdemir, M. Koca, N.M. Pınar, B. Demirci, K. Yıldırım, O. Sytar, H. Turkez, and K.H.C. Baser. 2020. A caryophyllene oxide and other potential anticholinesterase and anticancer agent in *Salvia verticillata* subsp. *amasiaca* (Freyn & Bornm.) Bornm. (Lamiaceae). J. Essent. Oil Res. 32(6), 512-525. Doi: https://doi.org/10.1080/10412905.2020.1813212

Karemu, C.K., M.W. Ndung'u, and M. Githua. 2013. Repellent effects of essential oils from selected eucalyptus species and their major constituents against *Sitophilus zeamais* (Coleoptera: Curculionidae). Int. J. Trop. Insect Sci. 33(3), 188-194. Doi: https://doi.org/10.1017/S1742758413000179

Karimi, A., A. Krähmer, N. Herwig, J. Hadian, H. Schulz, and T. Meiners. 2020. Metabolomics approaches for analyzing effects of geographic and environmental factors on the variation of root essential oils of *Ferula assa-foetida* L. J. Agric. Food Chem. 68(37), 9940-9952. Doi: https://doi.org/10.1021/acs.jafc.0c03681

Kaur, A., S. Kaur, R. Jandrotia, H.P. Singh, D.R. Batish, R.K. Kohli, V.S. Rana, and N.A. Shakil. 2021. Parthenin—A sesquiterpene lactone with multifaceted biological activities: insights and prospects. Molecules 26(17), 5347. Doi: https://doi.org/10.3390/molecules26175347

Khursheed, A., M.A. Rather, V. Jain, A.R. Wani, S. Rasool, R. Nazir, N.A. Malik, and S.A. Majid. 2022. Plant based natural products as potential ecofriendly and safer biopesticides: a comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. Microb. Pathog. 173(Part A), 105854. Doi: https://doi.org/10.1016/j.micpath.2022.105854

Kim, S., J. Yoon, and J.-H. Tak. 2021. Synergistic mechanism of insecticidal activity in basil and mandarin essential oils against the tobacco cutworm. J. Pest Sci. 94, 1119-1131. Doi: https://doi.org/10.1007/s10340-021-01345-8

Koyama, S. and T. Heinbockel. 2020. The effects of essential oils and terpenes in relation to their routes of intake and application. Int. J. Mol. Sci. 21(5), 1558. Doi: https://doi.org/10.3390/ijms21051558

Le, N.V., L.N. Sam, L.T. Huong, and I.A. Ogunwande. 2022. Chemical compositions of essential oils and antimicrobial activity of *Piper albispicum* C. DC. from Vietnam. J. Essent. Oil Bear. Plants 25(1), 82-92. Doi: https://doi.org/10.1080/0972060X.2022.2032840

Luz, A.I.R., M.G.B. Zoghbi, and J.G.S. Maia. 2003. Os óleos essenciais de *Piper reticulatum* L. e *P. crassinervium* H.B.K. Acta Amaz. 33(2), 341-344. Doi: https://doi.org/10.1590/1809-4392200332344

Ma, S., R. Jia, M. Guo, K. Qin, and L. Zhang. 2020. Insecticidal activity of essential oil from *Cephalotaxus sinensis* and its main components against various agricultural pests. Ind. Crops Prod. 150, 112403. Doi: https://doi.org/10.1016/j.indcrop.2020.112403

Manosathiyadevan, M., V. Bhuvaneshwari, and R. Latha. 2017. Impact of insects and pests in loss of crop production: a review. pp. 57-67. In: Dhanarajan, A. (ed.), Sustainable agriculture towards food security. Springer, Singapore. Doi: https://doi.org/10.1007/978-981-10-6647-4_4

Mattar, V.T., J.L. Borioni, A. Hollmann, and S.A. Rodriguez. 2022. Insecticidal activity of the essential oil of *Schinus areira* against *Rhipibruchus picturatus* (F.) (Coleoptera: Bruchinae), and its inhibitory effects on acetylcholinesterase. Pestic. Biochem. Physiol. 185, 105134. Doi: https://doi.org/10.1016/j.pestbp.2022.105134

Mssillou, I., H. Saghrouchni, M. Saber, A.J. Zannou, A. Balahbib, A. Bouyahya, A. Allali, B. Lyoussi, and E. Derwich. 2022. Efficacy and role of essential oils as bioinsecticide against the pulse beetle *Callosobruchus maculatus* (F.) in post-harvest crops. Ind. Crops Prod. 189, 115786. Doi: https://doi.org/10.1016/j.indcrop.2022.115786

Müller, G.C., A. Junnila, J. Butler, V.D. Kravchenko, E.E. Revay, R.W. Weiss, and Y. Schlein. 2009. Efficacy of the botanical repellents geraniol, linalool, and citronella against mosquitoes. J. Vector Ecol. 34(1), 2-8. Doi: https://doi.org/10.1111/j.1948-7134.2009.00002.x

Muñoz-Acevedo, A., M.C. González, Y.S. De Moya, and J.D. Rodríguez. 2023. Volatile metabolites of *Piper eriopodon* (Miq.) C.DC. from Northern region of Colombia and assessment of in vitro bioactivities of the leaf essential oil. Molecules 28(6), 2594. Doi: https://doi.org/10.3390/molecules28062594

Murtaza, M., A.I. Hussain, G.M. Kamal, S. Nazir, S.A.S. Chatha, M. Asmari, J. Uddin, and S. Murtaza. 2023. Potential applications of microencapsulated essential oil components in mosquito repellent textile finishes. Coatings 13(8), 1467. Doi: https://doi.org/10.3390/coatings13081467

Nwosu, L.C. 2018. Maize and the maize weevil: advances and innovations in postharvest control of the pest. Food Qual. Saf. 2(3), 145-152. Doi: https://doi.org/10.1093/fqsafe/fyy011

Papanastasiou, S.A., C.S. Ioannou, and N.T. Papadopoulos. 2020. Oviposition-deterrent effect of linalool – a compound of citrus essential oils – on female Mediterranean fruit flies, *Ceratitis capitata* (Diptera: Tephritidae). Pest Manag. Sci. 76(9), 3066-3077. Doi: https://doi.org/10.1002/ps.5858

Peschiutta, M.L., F. Achimón, V.D. Brito, R.P. Pizzolitto, J.A. Zygadlo, and M.P. Zunino. 2022. Fumigant toxicity of essential oils against *Sitophilus zeamais* (Motschulsky)

(Coleoptera: Curculionidae): a systematic review and meta-analysis. J. Pest Sci. 95, 1037-1056. Doi: https://doi.org/10.1007/s10340-021-01457-1

Ram, K. and R. Singh. 2021. Efficacy of different fungicides and biopesticides for the management of lentil wilt (*Fusarium oxysporum* f. sp. *lentis*): management of lentil wilt. J. AgriSearch 8(1), 55-58. Doi: https://doi.org/10.21921/jas.v8i01.19565

Rees, D. 2007. Insects of stored grain: a pocket reference. Csiro Publishing, Collingwood, Australia. Doi: https://doi.org/10.1071/9780643094673

Ruiz-Vásquez, L., L. Ruiz, H.D. Caballero, W. Ruiz, M.F. Andrés, C.E. Díaz, and A. Gonzalez-Coloma. 2022. Antifungal and herbicidal potential of *Piper* essential oils from the Peruvian Amazonia. Plants 11(14), 1793. Doi: https://doi.org/10.3390/plants11141793

Salehi, B., Z.A. Zakaria, R. Gyawali, S.A. Ibrahim, J. Rajkovic, Z.K. Shinwari, T. Khan, J. Sharifi-Rad, A. Ozleyen, E. Turkdonmez, M. Valussi, T.B. Tumer, L. Monzote, M. Martorell, and W.N. Setzer. 2019. *Piper* species: a comprehensive review on their phytochemistry, biological activities and applications. Molecules 24(7), 1364. Doi: https://doi.org/10.3390/molecules24071364

Santana, A.I., R. Vila, S. Cañigueral, and M.P. Gupta. 2016. Chemical composition and biological activity of essential oils from different species of *Piper* from Panama. Planta Med. 82(11/12), 986-991. Doi: https://doi.org/10.1055/s-0042-108060

Silva, M.A., G.M. Passarini, L.N. Martinez, V.A. Facundo, C.G.B. Teles, and C.C. Kuehn. 2019. Chemical constituents and bioactivities of essential oils from the genus *Piper* (Piperaceae): a review. South Am. J. Basic Educ. Tech. Technol. 6(2), 776-817.

Smith, G.H., J.M. Roberts, and T.W. Pope. 2018. Terpene based biopesticides as potential alternatives to synthetic insecticides for control of aphid pests on protected ornamentals. Crop Prot. 110, 125-130. Doi: https://doi.org/10.1016/j.cropro.2018.04.011

Stejskal, V., T. Vendl, R. Aulicky, and C. Athanassiou. 2021. Synthetic and natural insecticides: gas, liquid, gel and solid formulations for stored-product and food-industry pest control. Insects 12(7), 590. Doi: https://doi.org/10.3390/insects12070590

Vasantha-Srinivasan, P., M. Chellappandian, S. Senthil-Nathan, A. Ponsankar, A. Thanigaivel, S. Karthi, E.-S. Edwin, S. Selin-Rani, K. Kalaivani, F. Maggi, and G. Benelli. 2018. A novel herbal product based on *Piper betle* and *Sphaeranthus indicus* essential oils: toxicity, repellent activity and impact on detoxifying enzymes GST and CYP450 of *Aedes aegypti* Liston (Diptera: Culicidae). J. Asia-Pac. Entomol. 21(4), 1466-1472. Doi: https://doi.org/10.1016/j.aspen.2018.10.008

Vásquez-Ocmín, P.G., J.-F. Gallard, A.-C. van Baelen, K. Leblanc, S. Cojean, E. Mouray, P. Grellier, C.A.A. Guerra, M.A. Beniddir, L. Evanno, B. Figadère, and A. Maciuk. 2022. Biodereplication of antiplasmodial extracts: application of the Amazonian medicinal plant *Piper coruscans* Kunth. Molecules 27(21), 7638. Doi: https://doi.org/10.3390/molecules27217638

Wang, B., J. Sui, B. Yu, C. Yuan, L. Guo, A.M. Abd El-Aty, and B. Cui. 2021. Physicochemical properties and antibacterial activity of corn starch-based films incorporated with *Zanthoxylum bungeanum* essential oil. Carbohydr. Polym. 254, 117314. Doi: https://doi.org/10.1016/j.carbpol.2020.117314

Wang, Y., L.-T. Zhang, Y.-X. Feng, S.-S. Guo, X. Pang, D. Zhang, Z.-F. Geng, and S.-S. Du. 2019. Insecticidal and repellent efficacy against stored-product insects of oxygenated monoterpenes and 2-dodecanone of the essential oil from *Zanthoxylum planispinum* var.

dintanensis. Environ. Sci. Pollut. Res. 26, 24988-24997. Doi: https://doi.org/10.1007/s11356-019-05765-z

Wei, X.-M., S.-S. Guo, H. Yan, X.-L. Cheng, F. Wei, and S.-S. Du. 2018. Contact toxicity and repellency of the essential oil from *Bupleurum bicaule* helm against two stored product insects. J. Chem. 2018(1), e5830864. Doi: https://doi.org/10.1155/2018/5830864

Whitehead, S.R. and M.D. Bowers. 2014. Chemical ecology of fruit defence: synergistic and antagonistic interactions among amides from *Piper*. Funct. Ecol. 28(5), 1094-1106. Doi: https://doi.org/10.1111/1365-2435.12250

Whitehead, S.R., C.S. Jeffrey, M.D. Leonard, C.D. Dodson, L.A. Dyer, and M.D. Bowers. 2013. Patterns of secondary metabolite allocation to fruits and seeds in *Piper reticulatum*. J. Chem. Ecol. 39, 1373-1384. Doi: https://doi.org/10.1007/s10886-013-0362-4

Yang, K., C.F. Wang, C.X. You, Z.F. Geng, R.Q. Sun, S.S. Guo, S.S. Du, Z.L. Liu, and Z.W. Deng. 2014. Bioactivity of essential oil of *Litsea cubeba* from China and its main compounds against two stored product insects. J. Asia-Pac. Entomol. 17(3), 459-466. Doi: https://doi.org/10.1016/j.aspen.2014.03.011

Yildirim, E., B. Emsen, and S. Kordali. 2013. İnsecticidal effects of monoterpenes on *Sitophilus zeamais* motschulsky (Coleoptera: Curculionidae). J. Appl. Bot. Food Qual. 86, 198-204. Doi: https://doi.org/10.5073/JABFQ.2013.086.027

Yuan, L., X. Yang, X. Yu, Y. Wu, and D. Jiang. 2019. Resistance to insecticides and synergistic and antagonistic effects of essential oils on dimefluthrin toxicity in a field population of *Culex quinquefasciatus* Say. Ecotoxicol. Environ. Saf. 169, 928-936. Doi: https://doi.org/10.1016/j.ecoenv.2018.11.115

Zhang, L.-L., Y. Chen, Z.-J. Li, G. Fan, and X. Li. 2023. Production, function, and applications of the sesquiterpenes valencene and nootkatone: a comprehensive review. J. Agric. Food Chem. 71(1), 121-142. Doi: https://doi.org/10.1021/acs.jafc.2c07543