



Volatile chemical composition and bioactivities from Colombian *Kyllinga pumila* Michx (Cyperaceae) essential oil

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ABSTRACT. The essential oil from the fresh leaves of *Kyllinga pumila* (Michx) was obtained by hydrodistillation and characterized by gas chromatography-mass spectrometry (GC-MS). Twenty-eight volatile compounds were identified, major constituents of the oil were Methyl E,E-10,11-epoxyfarnesoate (43.8%), β -elemene (12.5%), Z-caryophyllene (11.3%), germacrene D (7.1%) and E-caryophyllene (5.6%). Repellent and fumigant activities of the oil against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), were done using the area preference method. Additionally, we studied their antioxidant and phytotoxic effects. Essential oils exhibited a dose-dependent repellent activity, with values 90% at the applied concentration (0.01%), for both two and four hour's exposure. Essential oil from *K. pumila* showed 92% mortality at 500 $\mu\text{L L}^{-1}$ air against *T. castaneum* on 24 hours of exposure. The value LC_{50} was 153.4 $\mu\text{L L}^{-1}$. With moderate selective phytotoxic effects on *L. perenne* root growth ($\pm 70\%$ inhibition). *Kyllinga pumila* shows high antioxidant potential (91.5%), an effect that is comparable with ascorbic acid (92.9%) used as a standard. The results indicated that *K. pumila* essential oil could be a promising alternative to new natural antioxidants, repellents, and biocides.

Keywords: essential oils, repellent activity, fumigant, *Tribolium castaneum*, phytotoxic, DPPH.

Composição química volátil e bioatividades do óleo essencial do colombiano *Kyllinga pumila* Michx (Cyperaceae)

RESUMO. O óleo essencial das folhas frescas de *Kyllinga pumila* Michx., foi obtido por hidrodestilação e caracterizado por cromatografia gasosa acoplada a espectrometria de massas (CG-EM). O constituinte majoritário do óleo foi Metil E,E-10,11-epoxifarnesoato (43,8%), β -elemeno (12,5%), Z-caryophylleno (11,3%), germacreno D (7,1%), E-caryophylleno (5,6%). A atividade repelente e fumigante do óleo frente à *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) foi determinada pelo método da área de preferência. Também, foi estudado os efeitos antiferrugens e fitotóxicos. Óleos essenciais exibiram uma atividade repelente dependente da dose, com valores de 90% na concentração aplicada (0,01%), tanto para duas e quatro horas de exposição. O óleo essencial de *K. pumila* mostrou mortalidade de 92% a 500 $\mu\text{L L}^{-1}$ ar frente à *T. castaneum*, em 24 horas de exposição. O valor LC_{50} foi 153,4 $\mu\text{L L}^{-1}$. Com efeitos fitotóxicos seletivos moderado sobre o crescimento da raiz de *L. perenne* ($\pm 70\%$ de inibição). *Kyllinga pumila* mostra um elevado potencial antioxidante (92%), um efeito que é comparável com ácido ascórbico (95%) usado como padrão. Os resultados indicaram que o óleo essencial *K. pumila* poderia ser uma alternativa promissora para novos antioxidantes, repelentes e biocidas naturais.

Palavras-chave: Plantas ciperáceas, óleos essenciais, atividade repelente, fumigante, *Tribolium castaneum*, DPPH.

Introduction

The genus *Kyllinga* belongs to Cyperaceae family, distributed in tropical, subtropical, and warm temperate regions around the world. Consists of about 40 species that are distributed worldwide. *Kyllinga pumila* Michx, known in Colombia as 'estrellón', is a perennial plant, growing in North America, South America, Caribbean Islands and Africa (Simpson & Inglis, 2001). Except for reports on *K. erecta* Schum. (Mahmout, Bessiere, & Dolmazon, 1993); *K. brevifolia*

(Guilhon, Vilhena, Zoghbi, Bastos, & Rocha, 2008) and *K. odorata* (Tucker, Maciarello, & Bryso, 2006), no reports exist on the chemistry of the essential oils (EOs) from *K. pumila* Michx species. One objective of this paper was to determine the volatile chemical composition of the EOs from *K. pumila* growing in the Department of Bolivar, Colombia. We believe that the data obtained from this study could contribute to the taxonomic investigation of the genus and explore possible uses of added value.

Natural products from several plants species, such as essential oils have demonstrated to act as repellents (De Lira et al., 2015; Jaramillo-Colorado, Martelo, & Duarte, 2012), toxicants (Harraz et al., 2015) and antifeedants (Julio et al., 2014) against insects that attack stored products (Erland, Rheault, & Mahmoud, 2015; Rajendran & Sriranjini, 2008). Furthermore, they are known for their antimicrobial and antioxidant properties, and their use in the food industry has been widely described (Duarte, Luis, Oleastro, & Domingues, 2016).

In previous studies, oils isolated from *Triphasia trifolia* (Jaramillo et al., 2012), *Laurelia sempervirens*, *Origanum vulgare* (Kim et al., 2010), *Achillea* (Nenaah, 2014), among others, showed repellent activity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). This is one of the stored-product pests most widespread and destructive, causing damage to storage grains directly by feeding (Rees, 2004) and indirectly by the secretion of quinones that are responsible for human allergies (Hodges, Robinson, & Hall, 1996). Also, the increasing public concern over pesticide safety and possible environmental damage has resulted in rising attention to natural products as alternatives for the control of stored pests (Rajendran & Sriranjini, 2008). Terpenoids present in essential oils have shown strong repellent or insecticide activity, including hydrogenated monoterpenoids, such as thymol, α -pinene, carvacrol, myrcene, and oxygenated sesquiterpenes as caryophyllene oxide (Kim et al., 2010). Additionally, many phenolic compounds such as eugenol, estragole, anethole, carvacrol, thymol, coniferyl alcohol, etc., are widely distributed in the plant kingdom and constitute one of the most important classes of natural antioxidants (Amorati, Foti, & Valgimigli, 2013). However, the main compounds found in essential oils are not always responsible for the biological activities, for this reason is necessary the identification and detection of all compounds (Jaramillo-Colorado et al., 2012).

As part of our ongoing search to improved botanical biopesticides, the volatile chemical composition of the *K. pumila* essential oil has been analyzed in this study using GC-MS along with their repellent and fumigant effects against *T. castaneum*, antioxidant activity, and its phytotoxicity against *L. perenne*.

Material and methods

Plant material

The fresh flowers and leaves from *K. pumila* were collected on a farm, next to Maria La Baja, Bolivar

department, Colombia (9° 58' 52" N, 75° 17' 55" W) in April of 2014. The taxonomic characterization of the plant was carried out at the Institute of Biology, Faculty of Natural Sciences-University of Antioquia, Medellin, Antioquia, Colombia (Dr. F. J. Roldan Palacios, Herbarium University of Antioquia, HUA 185261).

Extraction of the essential oil

Extraction of essential oil from *K. pumila* was done according to European Pharmacopeia (European Pharmacopeia, 2008). Five hundred grams of fresh leaves chopped and 900 mL of distilled water were placed in a flask. After hydrodistillation for three hours in a modified Clevenger apparatus, the essential oil was separated by decantation and added Na_2SO_4 anhydrous (Panreac, USA). For GC analysis, 30 μL of essential oil was added to 1.0 mL of dichloromethane (Merck, Germany) and 1 μL of this solution was injected into the injection port.

Chromatography analysis

The chromatographic analysis of the essential oils was carried out in a gas chromatograph Agilent Technologies 4890D, equipped with an injection port split/splitless (230°C, split ratio 30:1) and a flame ionization detector (FID) (250°C). For the separation of the mixtures were used a capillary columns: HP-5 (30 m \times 0.32 mm i.d \times 0.25 μm df), stationary phase 5% diphenyl-95% polydimethylsiloxane (J & W Scientific, USA) and ZB-WAX (30 m \times 0.53 mm i.d \times 0.50 μm df), stationary phase polyethylene glycol (Phenomenex Inc, USA). Oven temperature was 50°C for 2 min and then continued at the rate of 5°C min^{-1} to 250°C (5 min). A carrier gas, helium, adjust to a rate of 1.160 mL min^{-1} with inlet pressure head of the column of 87.3 kPa. The identity of the components was assigned by comparison of their linear retention indices (LRI), relative to C7-C30 n-alkanes (Supelco, Bellefonte, PA, USA) compared with the literature reported (Adams, 1995; Davies, 1990), and GC-MS spectra of each GC component with those of standard substances, Wiley library data of the GC. High-purity gases for chromatography (helium and hydrogen grade 5.0, and zero air) were obtained from Linde (Cartagena, Colombia).

GC-MS analyses were carried out using a gas chromatograph Agilent Technologies 7890A Network GC (Palo Alto, California, USA) coupled to a mass selective detector (MSD) Agilent Technologies 5975 inert GC-MS system, equipped with an automatic

injector Agilent 4513A. For the separation of the essential oil was used a capillary column HP-5MS (30m x 0.25 mm id) coated with 5% diphenyl-95% polydimethylsiloxane (0.25 μ m phase thickness) (J & W Scientific, USA). The oven temperature programmed was 50°C (2 min), 50-250°C at 5°C min⁻¹, 250°C (5 min); the injector temperature was 230°C; the carrier gas was helium (quality grade 5.0, 99.99%) at 87 kPa (1.17 mL min⁻¹); the injection mode was split (ratio of 1:20); the sample volume injected was 1 μ L. The mass spectra were obtained by electron-impact ionization (EI) and energy of 70eV. The temperatures of the ionization chamber and transfer line were 230 and 280°C, respectively, and the acquisition mass range was 30–400 m/z.

Insects and bioassays

Were used adults of *T. castaneum* 5–7 days post-eclosion. Bioassays were carried out in the dark incubators at 28–30°C and 70–80% relative humidity (r.h) at the laboratory of Agrochemical Research of the University of Cartagena. *T. castaneum* were reared with Oat (*Avena sativa*).

Repellent activity

Repellent activity was as described by Tapondjou, Adler, Fontem, Bouda, and Reichmuth (2005). The experimental method was measured using the area preference method. Filter papers (Whatman No. '1, diameter 9 cm) were cut in half. The essential oil of *K. pumila* was dissolved in acetone. The concentrations chosen to evaluate the repellent were 0.0001, 0.001, 0.01, 0.1 and 1%. A volume of 0.5 mL of each essential oil solution was applied slowly and uniformly to one-half of each filter paper, the other half of the filter paper was treated with acetone as a control. The treated and control half discs were dried at room temperature to allow evaporation of the solvent. Treated and untreated halves were attached to their opposites using adhesive tape and placed in Petri dishes. Twenty adults (5–7 day old) of *T. castaneum* were released separately at the center of each filter paper disc. The dishes were then covered and transferred to an incubator at room temperature. The treatments were replicated four times, and the numbers of insects presented on the control (Nc) and treated (Nt) areas of the disks were recorded after 2 and four hours of exposition (Jaramillo-Colorado et al., 2012). Percentage repellency (PR) values were calculated as follows:

$$PR (\%) = [(N_c - N_t) / (N_c + N_t)] * 100$$

The results obtained were transformed into percentage repellency and analyzed by ANOVA and Student t test. Mortality rates were calculated using statistical formulas Abbott and Probit to determine the LC₅₀. A statistical software Version 2009 Biostat (AnalystSoft Robust Business Solutions, BioStat 2009) was used, with a level of the confidence interval of 5%. Four replicates for each analysis were performed.

Fumigant activity

The toxic effect of the essential oil from *K. pumila* was tested on *T. castaneum* adults. To determine the fumigant toxicity were used filter paper discs (Whatman N° 1, 2-cm diameter pieces), deposited at the bottom of petri dish covers (90 x 15 mm). These were impregnated with oil at doses calculated to give equivalent fumigant concentrations of 500, 350, 250, 150, 50 μ L of oil L⁻¹ air, respectively. Twenty adult insects (1 to 10-d-old) were introduced and tightly capped (replicated four times for each concentration). Was employed as a positive control, Pirilan 50EC, commercial pesticides, this contains as active ingredient methyl pirimiphos (organophosphorus pesticide). Mortality percentage was determined after 24 hours from the start of exposure (Negahban, Moharramipour, & Sefidkon, 2007).

Antioxidant activity

Was evaluated as a measure of the ability to scavenge radicals, by reacting DPPH· (1,1-diphenyl-2-picrylhydrazyl) radical (Sigma, USA), with potential antioxidants (essential oil) and ascorbic acid (standard substance) (Merck, Germany). Two milliliters of a 3.6×10^{-5} M ethanolic solution of DPPH was added to 50 μ L of an ethanolic solution of the antioxidant. The decrease in absorbance at 517 nm was recorded in an UV-Vis spectrophotometer for 16 min. Antioxidant activity is expressed as percentage inhibition, which corresponds to the amount of radical DPPH· offset by essential oils, (inhibition percentage of DPPH· radical, % I DPPH·), according to the following equation (Jaramillo-Colorado et al., 2012):

$$\% I DPPH = \left[\frac{Abs_0 - Abs_1}{Abs_0} \right] \times 100$$

Where Abs₀ is the absorbance of control (without test sample), and Abs₁ is the absorbance of the test samples at different concentrations. The antioxidant activity was measured at 0.1, 0.5, 1.0, 1.5, 2.0, and 2.5 mg mL⁻¹ of *K. pumila* extracts.

Phytotoxic activity

The experiments were conducted with *Lolium perenne* seeds. 2.5 cm diameter filter paper with 20 μL of the test compound ($10 \mu\text{g } \mu\text{L}^{-1}$ for extracts and $5 \mu\text{g } \mu\text{L}^{-1}$ for pure compounds) were placed on 12-well plates (Falcon), according to Martin et al. (2011).

Germination was monitored for six days and the rootlet/leaf length measured at the end of the experiment (25 plantlets randomly selected for each test and digitalized with the application ImageJ 1.43, <http://rsb.info.nih.gov/ij/>). This was performed a non-parametric analysis of variance (ANOVA) on radical length data. The juglone (JU) ($5 \mu\text{g } \mu\text{L}^{-1}$) was used as a positive control.

Results and discussion

Volatile chemical analysis of *Kyllinga pumila*

The essential oil of *K. pumila* was isolated from its aerial parts using hydrodistillation method (yield 0.39%) and analyzed using GC-FID and GC/MS techniques, to determine its qualitative and quantitative composition. Twenty-eight components were identified by GC/MS representing about 97% of the composition of *K. pumila* essential oil. Figure 1 shows the chromatographic profile of the volatile compounds.

Peak identification for the chromatogram appears in Table 1 and the structures of compounds can be seen in Figure 2. The detected analytes were listed according to their elution order on the HP-5 column. The majority components found in the essential oil were Methyl E,E-10,11-epoxyfarnesoate (2,6-nonadienoic acid, 9-(3,3-dimethyloxiranyl)-3,7-dimethyl, methyl ester, E,E) (43.8%), β -Elemene (12.5%), Z-caryophyllene (11.3%), germacrene D (7.1%) and E- caryophyllene (5.6%), 2-Z-6-E-farnesol (2.7%).

The composition of *K. pumila* essential oil obtained from plants collected in Maria la Baja, Colombia, reveals the predominance of sesquiterpenoids compounds, principally, E-E-10,11-Epoxy farnesenic acid methyl ester (juvenile hormone III, JH III). This is an insect juvenile hormone, is structurally-related sesquiterpenoids that regulate developmental processes such as metamorphosis and reproduction. Methyl farnesoate, the immediate biosynthetic precursor to JH III in insects, has been identified in *Cyperus iria* and *Cyperus aromaticus* (Toong, Schooley, & Baker, 1988; Bede, Goodman, & Tobea, 1999; Bede, Teal, Goodman, & Tobea 2001), species belongs to *Cyperaceae* family too.

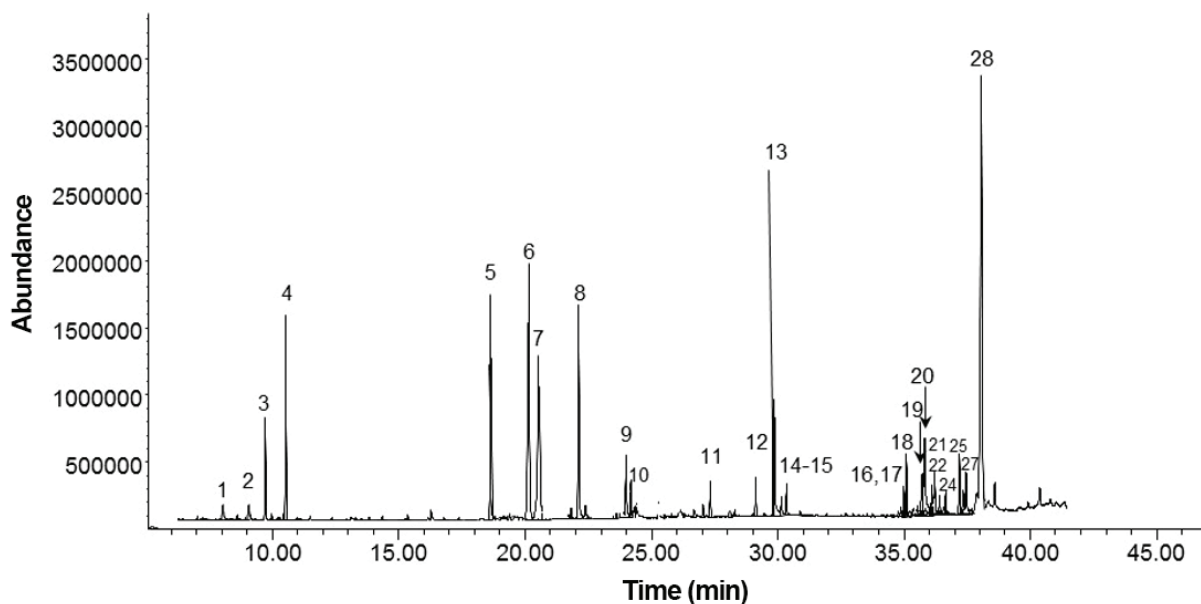
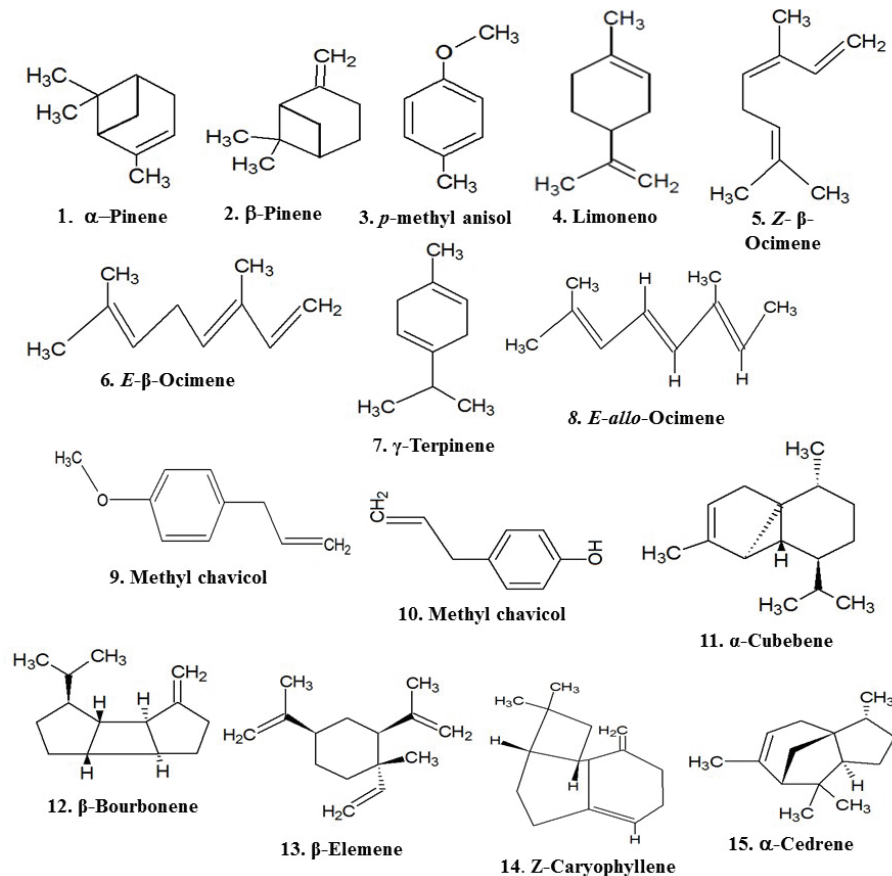


Figure 1. Typical chromatographic profile of the essential oil from Colombian *Kyllinga pumila* Michx. HP-5 column (30 m \times 0.32 mm i.d. \times 0.25 μm d_i), GC-FID. (See identification of number peak in Table 1).

Table 1. Volatile chemical composition of the essential oil from *Kyllinga pumila*, obtained by hydrodistillation.

Peak No ^a	Compound	LRI ^c HP-5	LRI ZB-WAX	Relative Area ^d , %
1	α -Pinene ^{b*}	941	1020	0.1 \pm 0.02
2	β -Pinene ^{b*}	985	1118	0.1 \pm 0.03
3	<i>p</i> -Methyl-anisol ^{b*}	1017	1430	0.2 \pm 0.07
4	Limonene [*]	1032	1216	0.5 \pm 0.04
5	<i>Z</i> - β -Ocimene ^{b*}	1038	1243	2.5 \pm 0.39
6	<i>E</i> - β -Ocimene [*]	1046	1230	2.9 \pm 0.95
7	γ -Terpinene ^{b*}	1060	1236	0.2 \pm 0.03
8	<i>E</i> - <i>allo</i> -Ocimene [*]	1130	1412	0.1 \pm 0.02
9	Estragol (methyl chavicol) ^{b*}	1189	1626	0.1 \pm 0.03
10	Chavicol ^{b*}	1250	2314	0.1 \pm 0.05
11	α -Cubebene ^{b*}	1351	1464	0.3 \pm 0.09
12	β -Bourbonene ^{b*}	1388	1518	1.3 \pm 0.18
13	β -Elemene ^{b**}	1391	1566	12.5 \pm 1.48
14	<i>Z</i> -Caryophyllene ^{b*}	1408	1577	11.3 \pm 0.89
15	α -Cedrene [*]	1409	1589	0.2 \pm 0.03
16	<i>E</i> -Caryophyllene ^{b*}	1420	1603	5.6 \pm 0.36
17	β -Cedrene [*]	1422	1615	0.4 \pm 0.09
18	<i>E</i> - β -Farnesene ^{b*}	1460	1659	1.5 \pm 0.45
19	<i>allo</i> -Aromadendrene [*]	1462	1660	0.2 \pm 0.03
20	Germacrene D ^{b*}	1478	1667	7.1 \pm 0.85
21	α -Selinene ^{b*}	1485	1692	0.4 \pm 0.10
22	(+) epi-Bicyclosesquiphellandrene [*]	1486	1712	1.3 \pm 0.63
23	α -Elemol [*]	1550	2025	0.5 \pm 0.09
24	Caryophyllene Oxide ^{b*}	1579	1988	0.9 \pm 0.16
25	2- <i>Z</i> , 6- <i>E</i> -Farnesol [*]	1684	2330	2.7 \pm 0.23
26	2- <i>E</i> , 6- <i>E</i> -Farnesol ^{b*}	1706	2349	0.3 \pm 0.02
27	Methyl Farnesate ^b	1758	2210	2.9 \pm 0.59
28	Methyl <i>E</i> , <i>E</i> -10,11-epoxyfarnesate (2,6-nonadienoic acid, 9-(3,3-dimethyloxiranyl)-3,7-dimethyl-, methyl ester, (<i>E</i> , <i>E</i>) <i>b</i>)	1806	2346	43.8 \pm 2.82

a) Peak number in Figure 1; b) Identification made by mass spectrum (EI: electron impact ionization, 70 eV; peak matching >90%) and LRIs. Spectral databases wiley8, NIST08; c) Experimentally LRI on the HP-5 and ZB-Wax column; d) Averages of three independent extractions; *Tentative identification based in LRIs on HP-5 column. LRI –Linear retention indices relative to C₇–C₃₀ n-alkanes.

**Figure 2.** Structures of principal compounds found in the essential oil from Colombian *Kyllinga pumila*. (See Table 1.) (continues...)

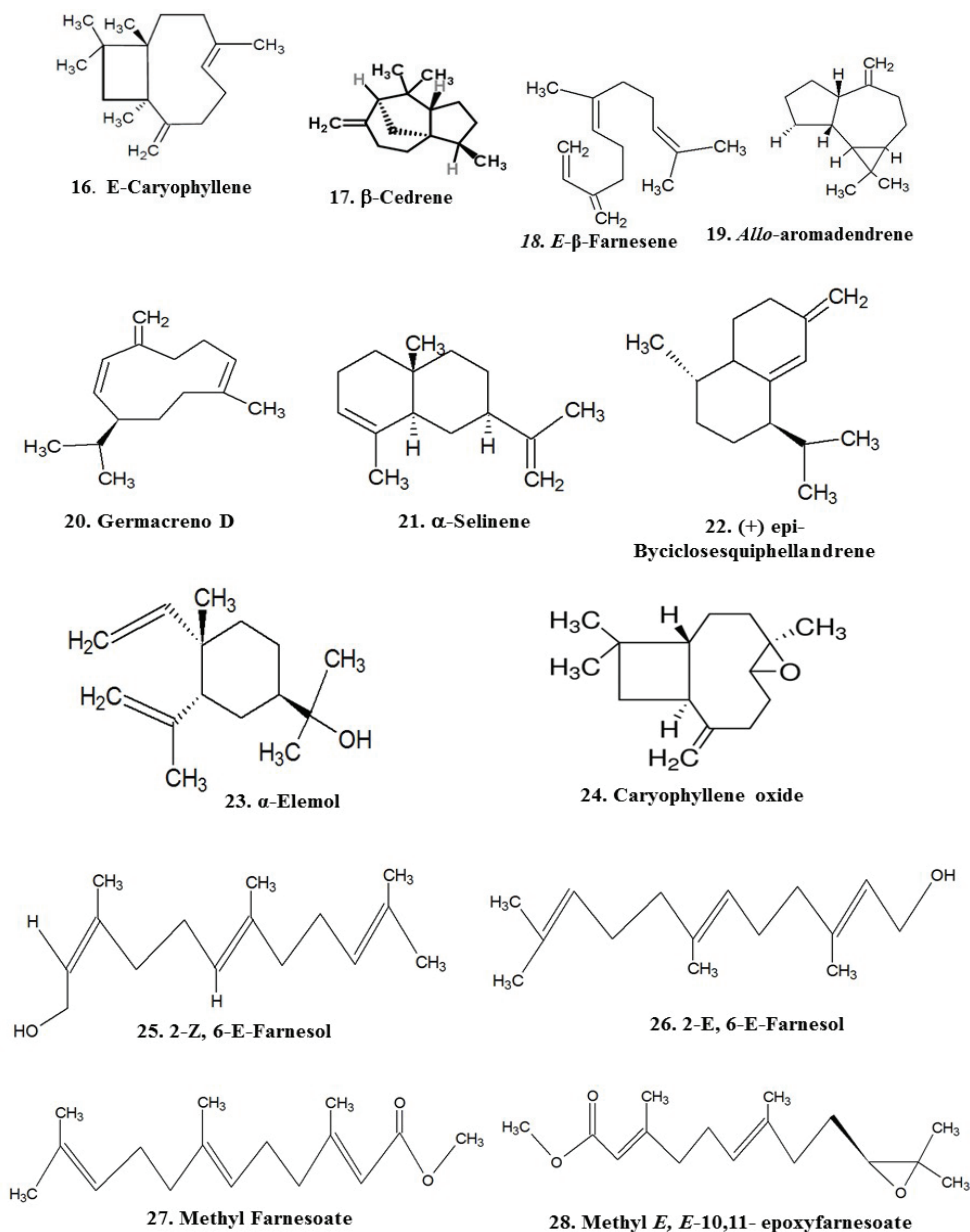


Figure 2 (cont.). Structures of principal compounds found in the essential oil from Colombian *Kyllinga pumila*. (See Table 1.)

There are not reports about the chemistry of the essential oils of *K. pumila* Mixch species. But exist publications related to *K. erecta* Schum., (Mahmout et al., 1993), *K. brevifolia* (Guilhon et al., 2008) and *K. odorata* (Tucker et al., 2006). The results obtained in those works were different compared with our study. The main components present in *K. brevifolia* were the manoyl oxide (6.8%-31.1%), 13-*epi*-manoyl oxide (5.7%-26.1%), 11 α -hydroxymanoyl oxide (5.9%-16.2%) and 1 β -hydroxymanoyl oxide (4.6%-22.1%). In *K. odorata* were dihydrokaranone (53.1 \pm 16.6%) and aristolochene (11.3 \pm 2.4%) (Tucker et al., 2006). And for *K. erecta*, Mahmout et al. (1993) reported manoyl oxide, cyprene,

sativene, and spathulenol). While Oyediji, Mdlolo, Adeniyi, & Akinde (2010) described 1,8-cineole (10.5%), α -humulene (21.7%), farnesyl acetate (11.2%).

Repellent activity

Kyllinga pumila oil showed significant pest repellent activity. The oil was repellent to *T. castaneum* adults at all concentrations (Table 2). *K. pumila* oil had a strong repellent activity to adults at a 0.01 $\mu\text{L cm}^{-2}$ and exposure period of two and four hours, repellency reached 90% for both. Thus, *K. pumila* oil has the potential for use with at least some stored-product insects as a repellent.

Table 2. Repellent activity of the essential oil from *Kyllinga pumila* against *Tribolium castaneum*.

Essential Oil	Concentration (%v v ⁻¹)	% Repellency as exposure time	
		2 hour	4 hours
<i>Kyllinga pumila</i> M.	10 ⁻⁴	45±2	50±5
	10 ⁻³	80±2*	75±5*
	10 ⁻²	90±2*	90±3*
	10 ⁻¹	75±5	75±2
	1	50±1	90±5

*Statistically significant difference between the number of organism in treated and untreated areas, using the paired t test (p < 0.001).

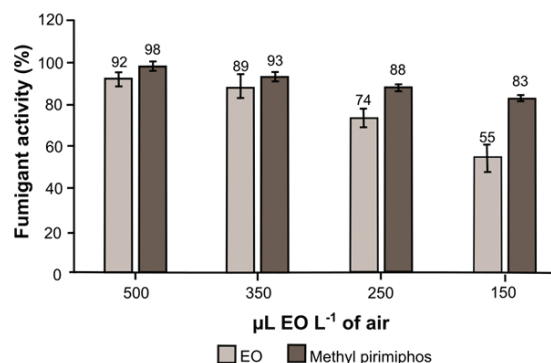
The insect known as weevil flour (*Tribolium castaneum*), is one of the most damaging for the food industry due to its high economic effect on flour, cereals, pasta, crackers, nuts, etc. (Nenaah, 2014). The essential oil of *K. pumila* had a high repellent activity (90,0%) against *T. castaneum*, this effect can be related to the primary compound found in the essential oil, JH III, as previously mentioned, play critical roles in physiological processes, such as metamorphosis and reproduction of the insects (Yang et al., 2013). Chaitanya, Sridevi, Senthikumar, & Dutta Gupta (2012) show that the insecticidal activity of JH III analogs may form part of a defensive strategy of plants against insect herbivores by preventing the development from insect larvae to insect adults. Furthermore, is well known that repellent properties of EOs are associated with the presence of specific compounds, specifically monoterpenoids and sesquiterpenes (Ukeh & Umoetok, 2011; Zhang et al., 2011; Tabanca et al., 2013). Zhang et al. (2011) showed that the essential oil of *Cymbopogon distans* aerial parts possessed strong repellency against the booklouse, *Liposcelis bostrychophila*, and the red flour beetle, *Tribolium castaneum*. Kim et al. (2010) evaluated the repellency activity against *T. castaneum* using nine constituents of *origanum* oil. Caryophyllene oxide and α -pinene produced the strongest repellency. These compounds were identified in the EO from Colombian *K. pumila*.

Fumigant activity

Results related to fumigant toxicity bioassays were shown in Figure 3. The values LC₅₀ and LC₉₅ for *K. pumilla* on *T. castaneum* were respectively 153.4 and 535.0 $\mu\text{L L}^{-1}$. Such activity seems to be moderate compared to Pirilan, methyl pirimiphos (commercial insecticide) with LC₅₀ and LC₉₅ values of 50.1 and 359.5 $\mu\text{L L}^{-1}$ air, respectively, as shown Table 3. The highest value of fumigant activity was 92.0 % at 500 $\mu\text{L EO L}^{-1}$ air (see Figure 3).

Essential oils of many plant species are insecticidal to stored-product insects (Rajendran & Sriranjini, 2008). The insecticidal propriety of many essentials oils is mainly attributed to

monoterpenoids which are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions (Suthisut, Fields, & Chandrapatya, 2011; Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Due to their high volatility, they have fumigant and gaseous action which are very crucial in controlling the stored-product insects (Bachrouh, Ferjani, Haouel, & Ben Jemaa, 2015).

**Figure 3.** Fumigant activity of essential oil (EO) from *Kyllinga pumila* against *Tribolium castaneum*.**Table 3.** Toxicity of essential oil from *Kyllinga pumila* and synthetic pesticide (Pirilan) against *Tribolium castaneum*.

Sample	Level Confidence Interval	LC		
		LC ₅₀ *	LC ₉₀ *	LC ₉₅ *
<i>Kyllinga pumila</i>	0.05	153.4±13	386.4±39	535.0±67
Pirilan, (methyl pirimiphos)	0.05	50.1±8.3	232.6±8.6	359.5±8.1

*LC₅₀, LC₉₀, LC₉₅ they are expressed in $\mu\text{L EO L}^{-1}$ of air.

Suthisut et al. (2011) found significative fumigant activity in individual monoterpenoids proved against *T. castaneum*. They were terpinen-4-ol, Camphor, isoborneol, 1,8-cineole, and β -pinene. These last has been known as possessing strong acetylcholinesterase inhibition activity from *T. castaneum* and *S. oryzae* (Zarrad, Ben Hamouda, Chaieb, Laarif, & Jemaa, 2015; Kim, Kang, & Park, 2013).

Antioxidant activity

Figure 4 shows that radical DPPH[•] was neutralized by the essential oil from *K. pumila*, with a maximum percentage of inhibition of 91.5% (2.5 mg mL⁻¹); a comparison was made with ascorbic acid (a substance used as a reference antioxidant), where the percentage of inhibition against DPPH[•] radical was 92.9%.

The DPPH radical is scavenged by antioxidants through the donation of hydrogen, which forms the reduced compound, DPPH-H. The color changes from purple to yellow after reduction (product known as diphenyl-picryl hydrazine), which can be

quantified by a decrease in the absorbance at 517 nm. The presence of an antioxidant leads to the disappearance of these radical chromogens (Jaramillo-Colorado et al., 2012). This resulting decolorization depends on the number of electrons that are captured. This scavenging occurs due to the donation of hydrogen ions as a result of the progression of the reaction between free radicals and antioxidants. Various works reported high scavenging activity of terpenes found in citrus peel essential oils as α -pinene, linalool, citronelol, myrcene, γ -terpinene, and limonene, among others (Behrendorff, Vickers, Chrysanthopoulos, & Nielsen, 2013; Sawamura, 2013). As antioxidant compounds donate a proton to the DPPH radical, greater weighting may be given to double bond positions that increase the availability of allylic protons (due to the weaker C-H bond at allyl groups) (Behrendorff et al., 2013).

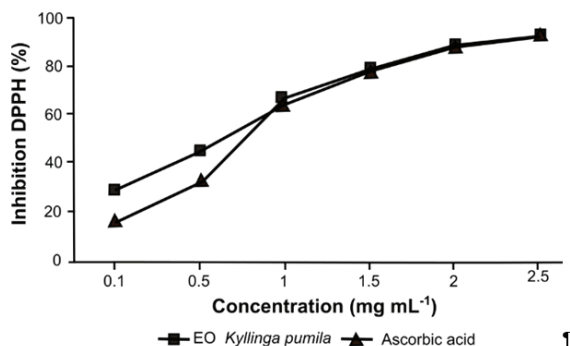


Figure 4. Measure of the ability to scavenge radicals, by reacting DPPH (1,1-diphenyl-2-picryl hydrazyl) radicals with essential oil of *Kyllinga pumila* and ascorbic acid (standard).

Phytotoxic activity

The effect of essential oil from *K. pumila* (100 $\mu\text{g cm}^{-2}$) was moderate and selective against the monocotyledonous weed *L. perenne*, and affected both root (70%) and leaf (78.2%) growth. The results can be checked in Table 4.

Table 4. Phytotoxic effects of *Kyllinga pumila* on *Lolium perenne*

	<i>Lolium perenne</i>	
	Growth (%C)	
<i>Kyllinga pumila</i> essential oil (100 $\mu\text{g cm}^{-2}$)	Radicular	Leaf
	70.0 \pm 0.8	78.2 \pm 2.2

%C: percentage of control. $p < 0.05$, Mann Whitney test.

Allelopathy is a process for which products of the secondary metabolism, as terpenes phenolic, of a certain vegetal intervene significantly, generally of antagonistic form, in the development of other species of plants. The volatile terpenes, components of essential oils, show important allelopathic action, as caryophyllene oxide, carvacrol, thymol, 1,8-

cineole, among others (Pinheiro et al., 2015; Oliveira, Moreira, & Mendes, 2013; Dias, Gomes, & Dallarmi, 2009).

Conclusion

This study demonstrated repellent and fumigant activities of *K. pumila* essential oils. This oil is promising and could be considered for practical applications for stored food pest control. This finding suggests that the EO of *K. pumila* or single active compound can be a source of potential candidates and precursors for the development of natural repellent or biocides and antioxidant products.

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