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CHEMICAL COMPOSITION, INSECTICIDAL, AND ANTIFUNGAL ACTIVITIES OF FRUIT ESSENTIAL OILS OF THREE COLOMBIAN *Zanthoxylum* SPECIES

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ABSTRACT

This study determined the chemical composition of essential oils isolated from *Zanthoxylum monophyllum* (Lam.) P. Wilson, *Z. rhoifolium* Lam., and *Z. fagara* (L.) Sarg. fruits by steam distillation, as well as testing antifungal and insecticidal activities of essential oils as potential pesticides. Gas chromatography-mass spectrometry (GC/MS) analysis identified 57 compounds. The main constituents in *Z. rhoifolium* oil were β-Myrcene (59.03%), β-phellandrene (21.47%), and germacrene D (9.28%), the major constituents of *Z. monophyllum* oil were sabinene (25.71%), 1,8-cineole (9.19%), and *cis*-4-thujanol (9.19%), whereas fruit oil of *Z. fagara* mainly contained germacrene D-4-ol (21.1%), elemol (8.35%), and α-cadinol (8.22%). *Zanthoxylum fagara* showed the highest activity on *Colletotrichum acutatum* Simmonds (EC_{50} 153.9 μL L^{-1} air), and *Z. monophyllum* was the most active against *Fusarium oxysporum* Schlechtend: Fr. f. sp. *lycopersici* (EC_{50} 140.1 μL L^{-1} air). *Zanthoxylum monophyllum* essential oil showed significant fumigant activity against *Sitophilus oryzae* (L.). This study demonstrated that *Zanthoxylum* essential oils exhibit important fungicidal activity on *F. oxysporum* and *C. acutatum*, which could become an alternative to synthetic fungicides to control plant fungal diseases, and *Z. monophyllum* oil is a potential fumigant against *S. oryzae*.

Key words: *Zanthoxylum monophyllum; Z. rhoifolium; Z. fagara*; *Sitophilus oryzae*; *Fusarium oxysporum* f. sp. *lycopersici; Colletotrichum acutatum*; fumigant, antifungal, insecticidal.

INTRODUCTION

Crop losses due to insects and plant diseases caused by fungi, bacteria, and viruses cause significant economic losses (Kotan *et al*., 2008; Kordali *et al*., 2008). Insect pests often cause extensive loss of products stored in tropical and semitropical environments (Isman, 2000). For example, *Sitophilus* species are serious cosmopolitan pests of stored grain (Liu and Ho, 1999). *Sitophilus zeamais* Motschulsky (maize weevil), *S. oryzae* L. (rice weevil), and *S. granaries* L. are the main representatives of this genus, which principally attack rice (*Oryza sativa* L.), maize (*Zea mays* L.), wheat (*Triticum sativum* Lam.), and sorghum (*Sorghum bicolor* (L.) Moench) among others, through direct feeding on grain kernels causing unfavorable effects on food quality, safety, and preservation (Huang *et al*., 1997; Tapondjou *et al*., 2002; Kim *et al*., 2003; Park *et al*., 2003; Arannilewa, 2007).

Harvest losses due to fungal disease in world crop production may amount to 12% or more in developing countries (Horbach *et al*., 2010). Many pathogens including *Fusarium oxysporum* (vascular wilt), *F. solani* (fruit rot) and *Colletotrichum gloeosporoides* (fruit rot) cause severe pre- and post-harvest damage to agriculture (Bajpai *et al*., 2008). *Fusarium* is a plant pathogen that causes wilt diseases of several economically important plants and is also known to produce toxins thought to contribute to wilting by affecting cell membrane permeability and disrupting cell metabolism (Garcés de Granada *et al*., 2001; Pawar and Thaker, 2007). *Colletotrichum* are pathogens that cause anthracnose in a wide range of woody and herbaceous crops. Symptoms are broad-ranging and include stem rot, dieback, and seedling blight. Fruits are affected during the pre- and post-harvest period (Roca *et al*., 2003; Muñoz *et al*., 2009).

Synthetic insecticides and fumigants are widely used to control grain pests and plant diseases. However, the indiscriminate application of synthetic products has led to various problems including toxic residues in treated products, environmental pollution, and resistance against

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pesticides by microorganisms and grain insect pests (Huang *et al*., 1997; Isman 2006; Bakouri *et al*., 2008; Kotan *et al*., 2008; Ye *et al.,* 2010). Therefore, because of increasing drawbacks of the continued use of conventional fumigants, an effort is needed to develop new alternative pesticides to replace those being currently used.

Essential oils are potential botanical sources of alternative compounds to fumigants being currently used because of their low toxicity for warm-blooded animals, high volatility and toxicity for stored grain pests and plant microorganisms (Lee *et al*., 2001; Abad *et al*., 2007). *Zanthoxylum* genus (Rutaceae) is made up of about 250 species of trees and shrubs in the world's tropical and temperate regions (Pirani, 1993). It is economically very important as a source of edible fruits, raw material for the cosmetics and perfume industries, as well as culinary applications. In Asia, *Z. bungeanum* Maxim. fruits are the most popular huajiao commercial product called "da hong pao" (big red robe). "Green huajiao", fruit of *Z. schinifolium* Siebold & Zucc. (Yang, 2008), is the other widely used spice in Sichuan. *Zanthoxylum* species have shown significant insecticidal and antifungal activity. The bark methanol extract of *Z. xanthoxyloides* caused significant mortality rates in *S. oryzae* and *Callosobruchus maculatus,* two stored-product insect pests (Owusu *et al*., 2007). *Zanthoxylum monophyllum* bark methanol extract showed significant activity against seven human pathogen fungi (Gómez *et al*., 2007). Ethanolic extracts of *Z. americanum* leaves, fruits, stem bark, and root demonstrated a broad spectrum of antifungal and antibacterial activity against *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Fusarium oxysporum* (Bafi-Yeboa *et al*., 2005).

In addition to culinary applications, many species of *Zanthoxylum* are used in traditional medicine especially in America, Africa, and Asia. *Zanthoxylum rhoifolium* Lam is popular in South America for inflammatory, microbial, cancerous, and malaria processes (Da Silva *et al*., 2007a; 2007b). *Zanthoxylum fagara* is used in Cuba for the treatment of diarrhea, chest diseases, intermittent fever, earaches, and tooth diseases (Dieguez-Hurtado *et al*., 2003). *Zanthoxylum monophyllum* is used as an analgesic to treat nasal inflammation, jaundice, and eye infections in Venezuela and as colorant (Cuca *et al*., 1998; Díaz and Ortega, 2006; Da Silva *et al*., 2007a).

Zanthoxylum species accumulate volatile oils in leaves, fruits, and inflorescences (Adesina, 2005). There are numerous reports on the chemical composition and the various biological activities of Zanthoxylum species essential oils (Choochote *et al*., 2007; Boehme *et al*., 2008; Yang, 2008). The chemical composition of essential oils of *Z. rhoifolium* flowers, fruits, and leaves (Gonzaga *et al*., 2003; Moura *et al*., 2006; Da Silva *et al*., 2007a; 2007b; Boehme *et al*., 2008), and biological properties of fruit and leaf essential oils, such as antibacterial and cytotoxic resultshave been previously reported (Moura *et al*., 2006; Da Silva *et al*., 2007b; Boehme *et al*., 2008).

The volatile chemical composition of *Z. monophyllum and Z. fagara* leaves from Costa Rica have also been reported (Setzer *et al*., 2005). However, there are no studies about their biological properties. The composition and biological properties of fruit essential oils of *Z. monophyllum* and *Z. fagara* have not yet been investigated.

We report the chemical composition, insecticidal activity against *S. oryzae*, and antifungal activity against *F. oxysporum* f. sp. *lycopersici* and *C. acutatum* of fruit essential oils of three Colombian plant species: *Z. monophyllum*, *Z. fagara,* and *Z. rhoifolium*.

MATERIALS AND METHODS

Plant material

The fruits of *Z. monophyllum* (4º11'24.3'' N, 74º30'48.9'' W) and *Z. fagara* (4º11'22.0'' N, 74º30'58.4'' W) were collected in January 2008 in the town of Icononzo, Tolima, Colombia, whereas *Z. rhoifolium* fruits (4º19'30.4'' N, 74º26'17.1'' W) were collected in February 2008 in the town of Fusagasugá, Cundinamarca, Colombia. Plant samples were identified by the Colombian National Herbarium of the Universidad Nacional de Colombia. Voucher specimens of *Z. monophyllum* (Lam.) P. Wilson (COL-517520), *Z. rhoifolium* Lam. (COL-522896), and *Z. fagara* (L.) Sarg. (COL-522891) were deposited in the Colombian National Herbarium of the Universidad Nacional de Colombia, Bogotá, Colombia.

Isolation of essential oils

Samples of fresh fruits (approximately 2 kg) of each *Zanthoxylum* species were submitted to steam distillation (*ca*. 4 h). Oils were dried over anhydrous sodium sulfate and stored at 0-5 ºC for further analysis.

Gas chromatography-flame ionization detector analysis (GC/FID)

Volatile compound analysis was performed with a gas chromatography system (Hewlett Packard 5890 GC) with a fused capillary column (50 m × 0.25 mm × 0.25 μm, HP-5MS, Crossbond 5% phenyl-95% dimethylpolysiloxane, Sigma-Aldrich, St. Louis, Missouri, USA) directly coupled to a flame ionization detector (FID). Injection conditions were the following: injector temperature at 250 ºC; oven temperature program at 45 ºC (2 min), 150 ºC (5 min) at a rate of 2 ºC min^{-1}, then at 150 ºC (2 min), 280 ºC (5 min) at a rate of 8 ºC min^{-1}; split 30:1 during 1.50 min, carrier gas He: 1 mL min^{-1}, constant flow; sample volume 1 μL.

Gas chromatography-mass spectrometry analysis (GC/MS)

The GC/MS analyses were performed in electronic impact (EI) mode with a Hewlett Packard-5890 GC system with a fused capillary column (50 m × 0.25 mm × 0.25 μm, HP-5MS, Crossbond 5% phenyl-95% dimethylpolysiloxane) directly coupled to a Hewlett Packard 5973 selective mass detector. Injection conditions were the same as described above. The mass spectrometer was operated at 70 eV.

Oil component identification

Oils were analyzed by GC-MS and GC with capillary columns (HP-5MS). Chemical constituents were identified based on the comparison of their mass spectral pattern and retention indices with those obtained from the Wiley 138.L, NBS 75K.L, and SDBS libraries, as well as those published by Adams (1995). Retention indices (RI) were calculated according to the literature (Van Den Dool and Kratz, 1963).

Insect rearing

Rice weevils were obtained from a colony maintained in the Laboratory of Entomology, Universidad Nacional de Colombia, Bogotá. Weevils were reared on maize grains. Cultures were maintained in the dark at 25 ± 1 °C and 70 ± 5% relative humidity.

Fungal cultures

Fusarium oxysporum f. sp. *lycopersici* was obtained from the culture collection of the Universidad de Cundinamarca (Laboratory of Phytopathology). *Colletotrichum acutatum* was obtained from the culture collection of the Universidad Nacional de Colombia, Bogotá (Laboratory of Vegetal Natural Products, Faculty of Science). Cultures were maintained and grown on potato dextrose agar (PDA) medium and incubated at 28 ± 1 °C.

Insecticidal activity assay

To determine the fumigant toxicity of *Zanthoxylum* oils, paper filter disks (Whatman Nº 1, 2-cm diameter pieces) were adhered to the inside of the Petri dish covers (90 x 15 mm) and then impregnated with oil at doses calculated to give equivalent fumigant concentrations of 242-967 μL L^{-1} air (20, 40, 60, and 80 μL oil). Twenty adult insects (1 to 10-d-old) were placed on each Petri dish. Phostoxin®-Fugran (phosphine - 300 μg L^{-1} air) and Nuvan® 50 E.C. (clorvox - 100 μL L^{-1} air) were used as positive controls. Petri dishes were sealed with Parafilm and incubated at 25 ± 1 °C and 70 ± 5% RH. Each concentration and control was replicated three times. Mortality was determined after 12, 24, and 48 h from the start of exposure (Negahban *et al*., 2007; Kotan *et al*., 2008). Insect mortality percentage (%M) was calculated by Abbott's correction formula (Pitasawat *et al*., 2007). LC_{50} and LC_{95} values, as well as the corresponding 95% confidence intervals, were estimated by probit analysis (Finney, 1971).

***In vitro* antifungal activity**

PDA plates were prepared with glass Petri dishes (90 x 15 mm) for the *in vitro* antifungal activity test. Agar plugs of actively growing cultures in PDA were placed on one half of the Petri dish (covered with PDA) and a sterilized paper disk was placed 2 cm from them. A 10 μL aliquot of each essential oil was added to the paper disks in each of the PDA plates (maximum 5 μL per disk). Plates were immediately sealed with Parafilm after adding each essential oil and incubated for 3 d at 28 °C. The diameter of concentric fungal mycelia was measured and compared with the untreated control. Medium effective concentration (EC_{50}) values were determined for essential oils that caused fungal growth inhibition. Aliquots of 2, 5, 7, 10, and 15 μL (23.5 - 176.5 μL L^{-1} air) of each essential oil were added to paper disks in each of the PDA plates (maximum 5 μL per disk). Plates were replicated three times in each treatment. (Lee *et al*., 2007; Kotan *et al*., 2008). In addition, Benlate 50WP (methyl-[1-[(butylamino) carbonyl]-1H-benzimidazol-2-yl] carbamate - 2 mg mL^{-1}) and Derosal®-Bayer (methyl-*1H*-benzimidazol-2-ylcarbamate - 1 mg mL^{-1}) were employed as chemical controls in the *F. oxysporum* f. sp. *Lycopersici* and *C. acutatum* assays, respectively.

Statistical analysis

Data are presented as mean ± standard error. Statistical significance was determined by the Duncan and Tukey tests; ANOVA determined whether results obtained for antifungal and insecticidal activity assays were statistically different. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Oil chemical composition

Fifty-seven compounds were identified by gas chromatography and mass spectrometry data in the fruit essential oils of three *Zanthoxylum* species (Table 1). Oils mainly contain monoterpenes and sesquiterpenes. Identified volatile components accounted for 89 to 99% of oil composition. Monoterpenes represent more than 70% of *Z. rhoifolium* oil composition (80.5%), *Z. monophyllum* (71.6%), and only 6.16% of *Z. fagara* oil composition. Sesquiterpenes represent 88.8% of *Z. fagara* oil composition. Chemical profiles obtained for these oils showed differences in composition among the studied species (Figure 1) although volatile constituents

Table 1. Chemical composition of *Zanthoxylum* species oils.

Peak	Constituents	RI[1]	Area		
			Z. rhoifolium	*Z. monophyllum*	*Z. fagara*
				%	
1	α-Thujene	929		1.6	
2	α-Pinene	937		3.1	2.9
3	Camphene	950			0.1
4	**Sabinene**	978		**25.7**	
5	β-Pinene	983			2.8
6	**β-Myrcene**	990	**59.0**	0.8	0.2
7	α-Phellandrene	1002		1.0	
8	α-Terpinene	1016		1.4	
9	**β-Phellandrene**	1034	**21.5**		0.2
10	**1,8-Cineole**	1037		**9.2**	
11	Z-β-Ocimene	1044		0.5	0.1
12	E-β-Ocimene	1053		0.7	
13	γ-Terpinene	1065		2.5	
14	**Trans-4-thujanol**	1072		**6.3**	
15	α-Terpinolene	1091		0.5	
16	**Cis-4-thujan**	1100		**9.2**	
17	2-Cyclohenen-1-ol	1125		0.6	
18	Terpinen-4-ol	1176		3.5	
19	α-Tepineol	1191		3.5	
20	Dihydroneoisocarveol	1229		1.2	
21	Sabinene hydrate acetate (trans)	1254		0.4	
22	Geraniol	1256			0.6
23	2-Undecanone	1294	1.7		
24	δ-Elemene	1337			0.4
25	α-Cubebene	1359		0.3	0.1
26	α-Copaene	1375		0.5	0.4
27	Geranyl acetate	1387			0.4
28	β-Elemene	1398	1.6		0.9
29	E-Caryophyllene	1423	1.6	1.0	2.7
30	γ-Elemene	1433			0.2
31	α-Caryophyllene	1453		0.4	1.5
32	Alloaromadendrene	1459			0.5
33	γ-Muurolene	1472			0.4
34	**Germacrene D**	1479	**9.3**	2.3	**6.0**
35	**Bicyclogermacrene**	1488	**3.1**	1.5	**5.8**
36	Germacrene A	1495	1.4		0.4
37	γ-Cadinene	1510			1.8
38	δ-Cadinene	1520		0.9	4.1
39	Cadina-1.4-diene	1529			0.4
40	α-Cadinene	1533			0.4
41	Elemol	1547		1.9	**8.4**
42	Germacrene B	1552		0.3	0.8
43	Trans-1-nerolidol	1560			0.5
44	**Germacrene D-4-ol**	1573		1.2	**21.1**
45	Guaiol	1601			0.9
46	10-α-Eudesm-4-en-11-ol	1623			0.8
47	γ-Eudesmol	1631			1.3
48	Hinesol	1637			3.2
49	**Tau-muurolol**	1640		1.6	**5.2**

Continuation Table 1.

Peak	Constituents	RI[1]	Area		
			Z. rhoifolium	*Z. monophyllum*	*Z. fagara*
			%		
50	Torreyol	1644		0.4	1.0
51	β-Eudesmol	1649		0.9	3.6
52	**α-Cadinol**	1654		**4.1**	**8.2**
53	**5-Neo-cedranol**	1680			**5.1**
54	Caryophyllene acetate	1701			0.6
55	E.E-Farnesol	1725		1.3	
56	α-bisabolol acetate	1794			0.2
57	E.E-farnesyl acetate	1850			0.3
	Monoterpenes	---	80.5	71.6	6.2
	Sesquiterpenes	---	18.7	18.2	88.8
	TOTAL	---	99.21	89.84	94.96

[1]Calculated retention index.

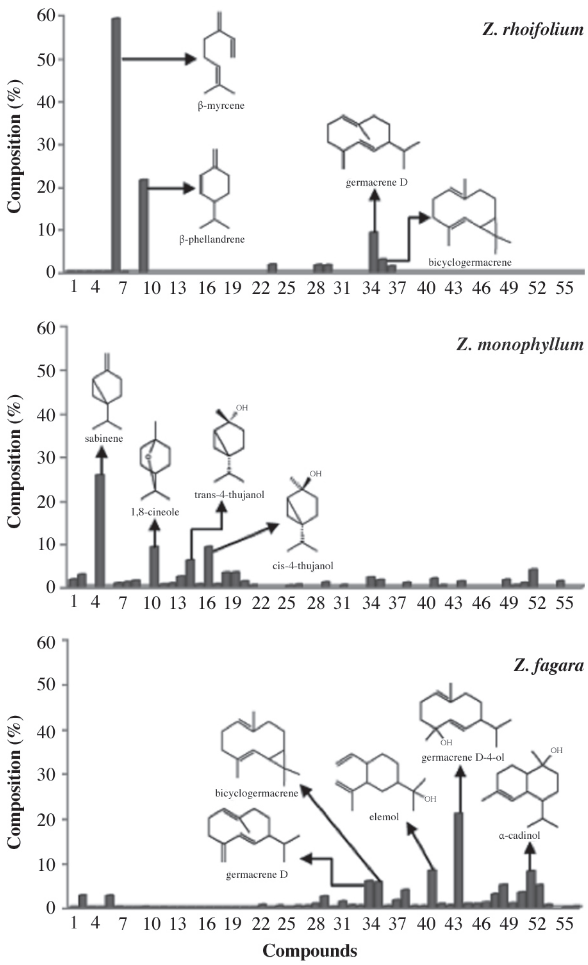

Figure 1. Chemical profile of essential oils from *Zanthoxylum* species fruits. For the key to identify peaks, see Table 1.

have been previously reported in other species of the *Zanthoxylum* genus (Moura *et al*., 2006).

The main constituents found in *Z. rhoifolium* fruit oil were β-myrcene (59.03%), β-phellandrene (21.47%), germacrene D (9.28%), and bicyclogermacrene (3.13%). Approximately 99.2% of *Z. rhoifolium* oil composition was characterized. The remaining unidentified components were mainly sesquiterpenes. The abundance of monoterpenoid and sesquiterpenoid compounds in fruit essential oil is in accordance with one previous report (Gonzaga *et al*., 2003); however, oil composition described in this study was qualitatively and quantitatively different, particularly regarding the major constituents. This suggests a considerable variability in the studied oil samples because of the influence of the ecological and chemical environment of each species, which affects the presence and abundance of secondary metabolites (Spitaler *et al*., 2006). The major constituents of oil derived from fruits collected in Brazil were reported as menth-2-en-1-ol (46.2%), β-myrcene (30.2%), (-)-linalool (15%), and terpineol (8.45%).

The major constituents identified in *Z. monophyllum* fruit oil were sabinene (25.71%), 1,8-cineole (9.19%), *trans*-sabinene hydrate (9.19%), and *cis*-sabinene hydrate (6.25%). *Zanthoxylum fagara* fruit oil mainly contained germacrene D-4-ol (21.1%), elemol (8.35%), α-cadinol (8.22%), germacrene D (5.96%), bicyclogermacrene (5.75%), *epi*-α-muurolol (5.15%), and 5-*neo*-cedranol (5.12%). Approximately 89.94% of *Z. monophyllum* and 94.2% of *Z. fagara* oil compositions were characterized. The remaining unidentified components were monoterpenes and sesquiterpenes. The detailed composition of fruit essential oils of *Z. fagara* and *Z. monophyllum* are reported for the first time in this study.

Insecticidal activity of the oils

Results show that the essential oils of *Z. fagara, Z. rhoifolium*, and *Z. monophyllum* have different insecticidal activity against *S. oryzae* adults. Insecticidal activity rises by increasing the dose and exposure times (Figure 2). *Zanthoxylum monophyllum* essential oil showed the best fumigant activity against rice weevil, *Z. rhoifolium* oil had weak fumigant toxicity while *Z. fagara* essential oil was inactive (Figure 2).

Zanthoxylum monophyllum essential oil caused significant mortality (about 90 to 99%) at 976 μL L^{-1} air dose after 24 h exposure. After 48 h of treatment, a 484 μL L^{-1} air dose is enough to cause 90% insect mortality. Fumigant activity of *Z. monophyllum* can be attributed to 1,8-cineole, terpinen-4-ol, and α-terpinene present in the essential oil; these compounds have shown 100% mortality on insects of the *Sitophilus* genus after 12 h exposure (Lee *et al*., 2004; Kordali *et al*., 2006).

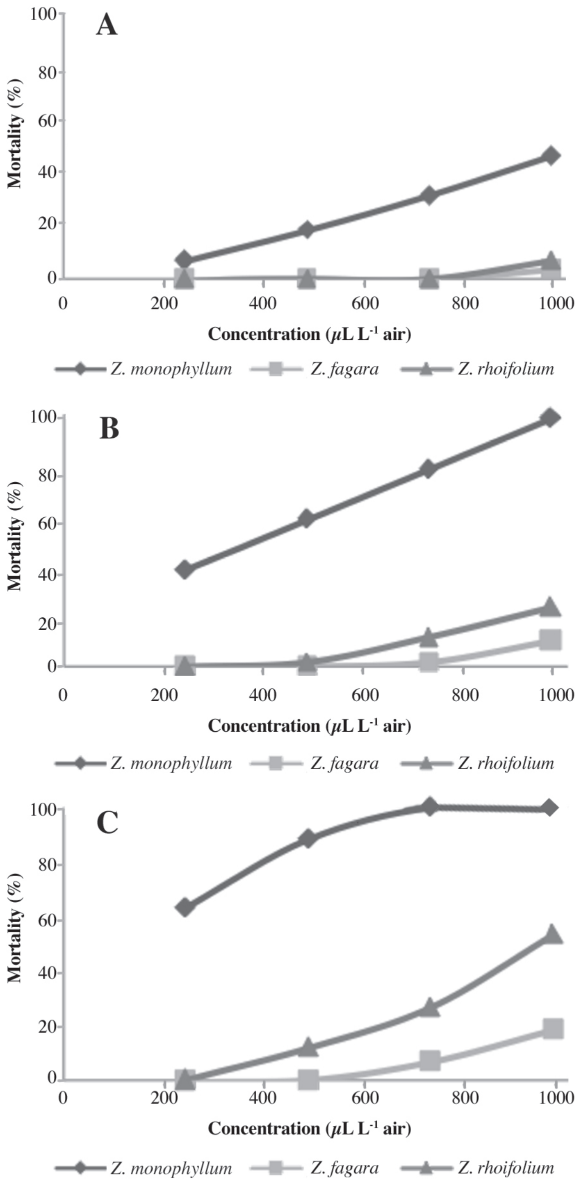

Figure 2. Percentage mortality of *Sitophilus oryzae* exposed to *Zanthoxylum* species oils for various time periods. A 12 h, B 24 h, and C 48 h.

The commercial fumigants Phosphamin (100 μg L^{-1} air) and Nuvan 50 (50 μL L^{-1} air) showed 100% mortality before 12 h exposure. *Zanthoxylum monophyllum* essential oil was less active than commercial products. Higher concentrations of essential oil or longer exposure times are necessary to obtain effects similar to those of commercial fumigants (Table 2).

Terpinen-4-ol, 1,8-cineole, and α-terpinene are absent in *Z. rhoifolium* and *Z. fagara* essential oils. Nevertheless, *Z. rhoifolium* shows insecticidal activity, which suggests that this activity can be due to the main oil components (β-myrcene and β-phellandrene) or to a possible synergistic effect of the compounds in the essential oil.

Oil antifungal activity

Essential oils were also evaluated as fumigants against two phytopathogenic fungi, *F. oxysporum* f. sp *lycopersici* and *C. acutatum*, which resulted in significant antifungal activity against both. For doses greater than 7 μL per Petri dish, antifungal activity of essential oils was found to be similar to or greater than the positive controls: benomyl (37.4 ± 0.10% inhibition growth) for the *F. oxysporum* f. sp *lycopersici* assay and carbendazym (29.9 ± 0.13% inhibition growth) for the *C. acutatum* assay (Figure 3).

Zanthoxylum fagara showed the highest antifungal activity on *C. acutatum* (EC_{50} 153.9 μL L^{-1} air) and *Z. monophyllum* was the most active against *F. oxysporum* f. sp *lycopersici* (EC_{50} 140.1 μL L^{-1} air) (Table 3). Minor components of *Z. fagara* were (E)-caryophyllene, T-muurolol, and α-cadinol (in a concentration of 2.7, 5.2, and 8.1% respectively) and *Z. monophyllum* (in a concentration of 1.0, 1.6, and 4.1% respectively) have shown antifungal activity against various phytopathogenic fungi including the *Fusarium* and *Colletotrichum* species when they were assayed as pure compounds. These results suggest that *Z. fagara* and *Z. monophyllum* activity can be attributed to the presence of these compounds (Chang *et al*., 2008).

Zanthoxylum rhoifolium essential oil has β-myrcene as its main component (59.0%), which is a compound that has shown antifungal activity in previous studies (Chang *et al*., 2008). This result suggests that moderate activity of the oil may be due to the presence of β-myrcene or the mixture of this compound with other metabolites that are toxic to the insect and present in the oil.

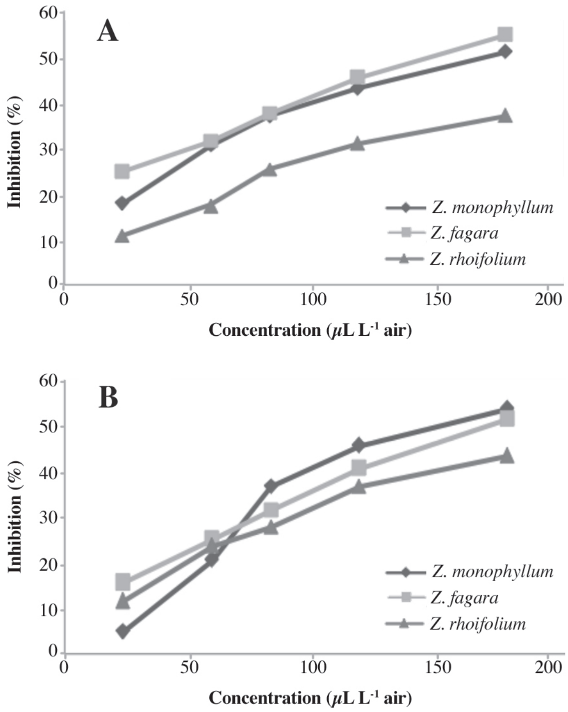

Figure 3. Concentration-dependent effects of *Zanthoxylum* species essential oils on mycelial growth of phytopathogenic fungi. A *Colletotrichum acutatum*, B *Fusarium oxysporum* f. sp. *lycopersici*.

CONCLUSIONS

We hoped to find new natural products for biocontrol of two important phytopathogenic fungi and stored-grain insect pest. The development of natural pesticides would also help to decrease the negative impact of synthetic agents, such as residues, resistance, and environmental pollution. In conclusion, fruit essential oils of *Z. monophyllum, Z. fagara*, and *Z. rhoifolium* could be recommended as fumigants against *F. oxysporum* and *C. acutatum* and as alternatives to synthetic fungicides in agriculture. Fruit essential oil of *Z. monophyllum* also showed an important insecticidal activity against *S. oryzae* and could be proposed as an important alternative insecticide to control this pest. It is important to note that *Z. fagara* essential oil was inactive against *S. oryzae*, but presented significant antifungal activity against two phytopathogenic fungi, a result that shows the selectivity of this essential oil against the phytopathogenic fungi species and the pest insect used in the bioassays. However, further studies are required to determine the cost, applicability, and safety of these oils as potential pesticides; it should be determined which of the secondary metabolites present in essential oils are responsible for antifungal and/or insecticidal activities, and then to establish if these substances are produced by plants regardless of their ecological environment.

Table 2. Fumigant toxicity of *Zanthoxylum monophyllum* and *Z. rhoifolium* essential oils against *Sitophilus oryzae*.

Plant species	Exposure time h	LC_{50} (95% FL[a]) μL L^{-1} air	LC_{95} (95% FL[1]) μL L^{-1} air
Zanthoxyllum rohifolium	12	> 967	--
	24	> 967	--
	48	944.3 (843.6-1027.7)	--
Zanthoxyllum monophyllum	12	> 967	--
	24	337.2 (295.6-367.8)	1122.2 (1088.2-1226.5)
	48	222.0 (173.5-259.9)	544.5 (491.6-640.2)

[1]Fiducial limits.

Table 3. Fumigant toxicity of *Zanthoxylum* species oils against *Colletotrichum* sp. and *Fusarium oxysporum*.

Plant species	EC_{50} (μL L^{-1} air) *Fusarium oxysporum* f. sp. *lycopersici*	EC_{50} (μL L^{-1} air) *Colletotrichum acutatum*
Zanthoxyllum fagara	182.6	153.9
Zanthoxyllum rohifolium	244.9	362.3
Zanthoxyllum monophyllum	140.1	172.8

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RESUMEN

Composición química, actividades insecticida y antifúngica de aceites esenciales de frutos de tres especies *Zanthoxylum* de Colombia. En este estudio se determinó la composición química de los aceites esenciales de frutos de *Zanthoxylum monophyllum* (Lam.) P. Wilson, *Z. rhoifolium* Lam. y *Z. fagara* (L.) Sarg. obtenidos mediante destilación por arrastre con vapor y se evaluó la actividad antifúngica e insecticida de los aceites esenciales para estimar su uso como posibles plaguicidas. El análisis por cromatografía de gases-espectrometría de masas (CG/EM) permitió la identificación de 57 compuestos. β-*Mirceno* (59,03%), β-*felandreno* (21,47%) y germacreno D (9,28%) fueron los componentes principales del aceite de *Z. rhoifolium*; los principales componentes del aceite de *Z. monophyllum* fueron sabineno (25,71%), 1,8 -cineol (9,19%) y cis-4-thujanol (9,19%), mientras que el aceite de frutos de *Z. fagara* está compuesto principalmente por germacreno D-4-ol (21,1%), elemol (8,35%) y α-cadinol (8,22%). *Zanthoxylum fagara* presentó la mayor actividad sobre *Colletotrichum acutatum* Simmonds (EC_{50} 153,9 μL L^{-1} de aire) y *Z. monophyllum* fue el más activo contra *Fusarium oxysporum* Schlechtend: Fr. f. sp. *lycopersici* (EC_{50} 140,1 μL L^{-1} de aire). El aceite esencial de *Z. monophyllum* mostró actividad insecticida significativa contra *Sitophilus oryzae* (L.) Este estudio demuestra que los aceites esenciales de *Zanthoxylum* poseen importante actividad antifúngica sobre *F. oxysporum* y *C. acutatum* y podría convertirse en una alternativa frente a los fungicidas sintéticos empleados comúnmente para el control de enfermedades de las plantas, así como el aceite de frutos de *Z. monophyllum* tiene un potencial para ser utilizado como fumigante contra *S. oryzae*.

Palabras clave: *Z. monophyllum; Z. rhoifolium; Z. fagara*; *Sitophilus oryzae*; *Fusarium oxysporum* f. sp. *lycopersici; Colletotrichum acutatum*; fumigante, antifúngicos, insecticida.

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