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Research Article

Chemical Composition of Essential Oils from Six *Zanthoxylum* Species and Their Repellent Activities against Two Stored-Product Insects

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The objective of this study was to analyze six essential oils from *Zanthoxylum* genus (family Rutaceae) in China and evaluate their repellent activities against *Tribolium castaneum* and *Lasioderma serricorne* adults. Six essential oils from *Zanthoxylum* genus, including *Z. armatum*, *Z. dimorphophyllum*, *Z. dimorphophyllum* var. *spinifolium*, *Z. piasezkii*, *Z. stenophyllum,* and *Z. dissitum,* were obtained by hydrodistillation and their yields were ranging from 0.02% to 0.53%. Totally, there were 39 chemical components revealed by GC-MS. Among them, some components with high relative content existed in more than three *Zanthoxylum* species. For instance, both δ-cadinene (1.21%–17.15%) and spathulenol (0.36%–10.19%) appeared in essential oils of *Z. dimorphophyllum*, *Z. piasezkii*, *Z. stenophyllum,* and *Z. dissitum* which were found to have higher content of sesquiterpenoids. The repellent activities of six essential oil samples against *T. castaneum* and *L. serricorne* adults were investigated for the first time. Data demonstrated that six *Zanthoxylum* species had much stronger repellent activities against *T. castaneum* than *L. serricorne* adults, especially in 2 hours after exposure. The results indicate that these six essential oils from *Zanthoxylum* have significant potential to be developed into natural repellents to control insects in grains, food, and traditional Chinese medicinal materials.

1. Introduction

Tribolium castaneum, the red flour beetle, and *Lasioderma serricorne*, the cigarette beetle, are two major destructive primary agricultural pests of stored grains, cereal foods, or traditional Chinese medicinal materials in both tropics and subtropics [1–3]. At warm and humid facilities, both of them can build up to huge populations very quickly. It was reported that these two stored insects could not only cause substantial quantitative and qualitative losses of stored commodities but also result in temperature raising and humidity conditions which lead to a promoted growth of harmful spoilage bacteria, molds, including toxigenic species. In recent years, many attempts, such as the hot air, the ionizing radiation, the cold storage, and the synthetic chemicals, have been made to control pests in storage. Among them, the heavily use of synthetic chemicals is the most popular method in controlling pests in storage. However, long time use of synthetic chemicals has led to a several of undesirable effects, including possible hazards to nontarget animals, risk to environmental pollution, and development of resistance by insects' resurgence [4–6]. Thus, finding out alternative ecologically safe, biodegradable, convenient, less persistent, low-cost, more pest specific methods to control stored insect is becoming more and more urgent. Recently, researchers have provided a novel conception of antagonistic storage, which could be tracing back to Ming dynasty of China [7]. It is a method using odorous Chinese herbs to store with medicinal herbs, which is vulnerable to insects, to avoid infestations and mildew and keep traditional Chinese

Table 1: Collecting information of the six *Zanthoxylum* species.

Species	Date	Sample mass (Kg)	Essential oil volume (mL)	Yield (V/W%)	Voucher specimens
Zanthoxylum armatum DC.	2015.09	1.60	8.48	0.53	BNU-CMH-DuSS-2015-09-02-001
Zanthoxylum dimorphophyllum Hemsl.	2015.09	0.40	0.52	0.13	BNU-CMH-DuSS-2015-09-02-002
Zanthoxylum dimorphophyllum Hemsl. var. *spinifolium* Rehder et Wils.	2015.09	0.60	1.68	0.28	BNU-CMH-DuSS-2015-09-02-003
Zanthoxylum dissitum Hemsl.	2015.09	1.10	0.55	0.05	BNU-CMH-DuSS-2015-09-02-006
Zanthoxylum piasezkii Maxim.	2015.09	0.52	2.50	0.48	BNU-CMH-DuSS-2015-09-02-004
Zanthoxylum stenophyllum Hemsl.	2015.12	3.00	0.60	0.02	BNU-CMH-DuSS-2015-12-02-005

medicinal herbs in good color. Therefore, as the secondary metabolism of aromatic plants, volatile essential oils have drawn more and more attention. Currently, a great number of essential oils from natural plants have been investigated, and some of them have turned out to possess strong insecticidal, repellent, and feeding deterrent effects [8–11].

Zanthoxylum is the largest and the most widespread genus in the Rutaceae, comprising approximately 250 species, and is widely distributed in the temperate and tropical regions, of which there are 45 species and 13 varieties in China [12, 13]. Traditionally, members of them with pungent taste are often used as condiments in both Eastern Asian countries and Central America [14]. Also, *Zanthoxylum* species have a folklore history in the treatment of various diseases, such as inflammation [15], toothache, lumbago [16], ascarid infection [17], sickle-cell anaemia, and malaria. Literatures have also shown that *Zanthoxylum* species possess a potential anti-insect [7, 18, 19], antimicrobial [20], antibacterial [21], and antifungal [22] activities.

To inherit and develop Chinese traditional method of antagonistic storage, the repellent activities against *T. castaneum* and *L. serricorne* of essential oils from six *Zanthoxylum* species, including *Zanthoxylum armatum*, *Zanthoxylum dimorphophyllum*, *Zanthoxylum dimorphophyllum* var. *spinifolium*, *Zanthoxylum piasezkii*, *Zanthoxylum stenophyllum,* and *Zanthoxylum dissitum* were investigated for the first time, and their chemical composition was evaluated as well. What is more, the chemical composition of essential oils from *Z. dimorphophyllum*, *Z. piasezkii,* and *Z. stenophyllum* was reported for the first time.

2. Experimental

2.1. Experimental Samples and the Extraction of Essential Oils. The experimental samples were collected from mature plants in Wen County (32.59N, 104.69E) of Gansu province, China. All the plant samples were identified by Professor Liu, Q. R. (College of Life Sciences, Beijing Normal University, Beijing, China) and voucher specimens in Table 1 were deposited at the herbarium (BNU) of Faculty of Geographical Science, Beijing Normal University. The branches and leaves of the six *Zanthoxylum* species were air dried and grounded to powders. The six sample powders were subjected to hydrodistillations using a modified Clevenger type apparatus as we described in our recent investigation for 6 hours, respectively [23]. Anhydrous sodium sulfate was used to remove extra water. Then, the six essential oil samples were stored in dark airtight containers in a refrigerator at 4°C.

2.2. Insects. *Tribolium castaneum* and *Lasioderma serricorne* were identified by Professor Liu, Z. L. (College of Plant Protection, China Agricultural University, Beijing, China). Culture of the red flour beetles and the cigarette beetle followed the same method mentioned in our recent literature [24]. Both of them were obtained from laboratory cultures maintained in the dark in incubators at 29–30°C and 70%–80% relative humidity (RH). They were reared in glass containers (0.5 L) containing wheat flour mixed with yeast (10 : 1, w/w) at 12%-13% moisture content. The unsexed adults used in all the experiments were about 7 ± 2 days old. All culture containers used in experiments were made escape proof with a coating of polytetrafluoroethylene (Sino-rich®, Beijing Sino-rich Tech Co., Ltd., Xuanwu District, Beijing, China).

2.3. Gas Chromatography and Mass Spectrometry. GC analysis of six essential oil samples was performed by a Thermo Finnigan Trace DSQ GC/MS instrument (Thermo Finnigan, Lutz, FL, USA) equipped with a flame ionization detector (FID) and a capillary column of HP-5MS ($30\,m \times 0.25\,mm \times 0.25\,\mu m$). The mass spectrometer was operated in the electron-impact mode, with ionization energy of 70 eV in m/e ranging 10–550 amu. In GC-FID and GC-MS analysis, the same column and analysis conditions as described in our recent study [23] were used. The temperature was programmed isothermal at 50°C for 2 minutes, rising up from 50 to 150°C at the speed of 2°C/min, then held isothermal at 150°C for 2 minutes, rising up from 150 to 250°C at a high speed of 10°C/min, and finally was kept isothermal at 250°C for 5 minutes. The injector temperature was 250°C, and the flow rate of carrier gas (helium) was 1.0 mL/min. Samples were diluted in hexane and then injected in the split mode. Identification of components by gas chromatography/mass spectrometry is a good literature for retention indices of chemical components of essential oils [25]. Quantification was determined by percentage peak area calculation using GC-FID, and some chemical components were identified by coinjections with standard (wherever possible) and confirmed by using the National Institute of Standards and Technology (NIST) version 05 GC-MS libraries (Standard

Reference Data, Gaithersburg, MD, USA) and Wiley 275 mass-spectral libraries (Wiley, New York, NY, USA) or in the literature [26–28]. Relative percentages of each component in the essential oil samples were obtained by averaging the GC-FID peak area% reports.

2.4. Repellency Tests. A modified area preference method [24, 29] was performed to the repellent activity against *T. castaneum* and *L. serricorne* adults for all essential oil samples. The six testing essential oil samples were dissolved separately in *n*-hexane to five different testing concentrations (78.63, 15.73, 3.15, 0.63, and 0.13 nL/cm^2). As experimental containers, Petri dishes (9 cm in diameter) were used to housing *T. castaneum* and *L. serricorne* adults. All the Petri dishes were pretreated with polytetrafluoroethylene (Sino-rich®, Beijing Sino-rich Tech Co., Ltd., Xuanwu District, Beijing, China) on the wall to prevent insects from escaping during repellent test. Every filter paper (9 cm in diameter) used was cut into two equal pieces. One piece was treated with 500 μL of testing solution, while another piece was added with the same volume of *n*-hexane as blank control. After air drying for 30 s, the two pieces of filter paper were carefully fixed with solid glue on the bottom of a Petri dish side by side tightly. During each test, twenty insects were released at the center of the disk and then covered quickly with dish cover. Counting of insects was performed very carefully on each half piece of paper at 2 and 4 h after exposure, respectively. Five replicates were performed for each tested concentration and each test was repeated for three times. Meanwhile, N,N-diethyl-3-methylbenzamide, DEET, a commercial repellent purchased from Dr. Ehrenstorfer (Augsburg, Germany) was used as a positive control. For each essential oil sample, the value of percent repellency (PR) was calculated as follows:

$$\text{PR}\,(\%) = \left[\frac{(Nc - Nt)}{(Nc + Nt)}\right] \times 100, \quad (1)$$

Nc was the number of insects on the control half, while Nt was the number of insects on the opposite side of testing. Analysis of variance (one-way ANOVA with Tukey post hoc test) was conducted by using Origin 2016 software.

3. Results and Discussion

3.1. Chemical Composition of the Essential Oil. Chemical components of essential oils from six *Zanthoxylum* species, including *Z. armatum*, *Z. dimorphophyllum*, *Z. dimorphophyllum* var. *spinifolium*, *Z. piasezkii*, *Z. stenophyllum,* and *Z. dissitum* were presented in Table 2. The yields of essential oils obtained from six *Zanthoxylum* genus ranged from 0.02% to 0.53% (v/w%). Though there are similarities in some of essential oil samples, their main components were quite different. For instance, the major components of *Z. armatum* essential oil were β-terpinene (45.56%), piperitone (33.47%), and 3-carene (8.88%), whereas β-caryophyllene, caryophyllene oxide, spathulenol, and δ-cadinene were the principal constituents of *Z. dimorphophyllum* essential oil with the relative contents of 26.17%, 13.36%, 10.19%, and 8.58%, respectively. As one of the main compounds, safrole only appeared in the essential oil of *Z. dimorphophyllum* var. *spinifolium*, while, as one of common chemical compound, eucalyptol turned out to exist only in *Z. piasezkii* essential oil. α-Cubebene, as a main compound of *Z. stenophyllum*, also appeared in the essential oil of *Z. dimorphophyllum*. The major constituents of *Z. dissitum* volatile oil were γ-pyronene (21.97%), germacrene D (20.98%), δ-cadinene (17.15%), and α-farnesene (14.28%). Unlike the essential oil samples of *Z. dimorphophyllum*, *Z. stenophyllum,* and *Z. dissitum* consisting mostly of sesquiterpenoids, essential oils from *Z. armatum* and *Z. piasezkii* consisted mostly of monoterpenoids. Interestingly, as the lower taxa, the chemical components of essential oil from *Z. dimorphophyllum* var. *spinifolium* varies from *Z. dimorphophyllum*. Different original place and different harvest time might result in different chemical composition for the same plant essential oil. In one recent report [18], the main components from *Z. armatum* essential oil harvested in June in India were 2-undecanone (19.75%), followed by 2-tridecanone (11.76%) and β-caryophyllene (9.88%). What is more, different extract parts also resulted in different chemical component. A recent literature [30] showed that the chemical compounds of *Z. dissitum* essential oils extracted from leaves and roots were significantly different. The major compounds of essential oil from *Z. dissitum* leaves were δ-cadinol (12.8%), caryophyllene (12.7%), β-cubebene (7.9%), and 4-terpineol (7.5%), while the main components from *Z. dissitum* roots essential oil were humulene epoxide II (29.4%) and caryophyllene oxide (24.0%). *Z. dimorphophyllum* var. *spinifolium* leaves harvested in April in the same place as our sample turned out to have different chemical composition [31]. Unlike our study, the main compounds of the essential oil from *Z. dimorphophyllum* var. *spinifolium* leaves reported by them were myristicin (24.85%), safrole (20.47%), and methyl eugenol (19.76%). Despite these reports, there is the first report on chemical component analysis for *Z. dimorphophyllum*, *Z. piasezkii,* and *Z. stenophyllum*.

3.2. Repellent Activity. Among various investigated plants, the *Zanthoxylum* genus stands out for its extracts and essential oils exhibited insecticidal, fungicidal, antibacterial, and fumigant activities [16, 19, 22]. Here, we first report repellent activities of six *Zanthoxylum* species including *Z. armatum*, *Z. dimorphophyllum*, *Z. dimorphophyllum* var. *spinifolium*, *Z. piasezkii*, *Z. stenophyllum,* and *Z. dissitum* against two storage pests including *T. castaneum* and *L. serricorne* adults.

The results of the repellent activities of the essential oils from these six *Zanthoxylum* species were presented in Figures 1 and 2. It demonstrated that all essential oil samples exhibited obvious repellent activities against two stored insects. At tested concentrations of 78.63, 15.73, 3.15, and 0.13 nL/cm^2, all of the six volatile oils exhibited higher repellency than the positive control of DEET against *T. castaneum* at 2 hours after exposure. Even exposed for 4 hours, these six essential oils also showed high repellent activities against *T. castaneum*. Among these essential oil samples, *Z. armatum* essential oil turned out to be the most effective repellent against *T. castaneum* at both 2 and 4 h after exposure. This might be attributed to its high content of monoterpenoids, which account for 93.62% of total essential oil. Unlike the repellency on *T. castaneum*, data in Figure 2 showed that essential oils

Table 2: Chemical composition of essential oils from the six *Zanthoxylum* species.

Compound	Relative content (%)						RI[a]	RI[b]	Identification methods[c]
	Z. armatum	*Z. dimorphophyllum*	*Z. dimorphophyllum* var. *spinifolium*	*Z. piasezkii*	*Z. stenophyllum*	*Z. dissitum*			
Camphene	—	—	—	2.45	—	—	943	941	RI, MS
β-Thujene	—	—	—	3.56	—	—	969	965	RI, MS
β-Pinene	—	—	—	—	4.80	—	977	983	RI, MS, Co
3-Carene	8.88	—	—	0.62	8.30	—	1004	1006	RI, MS
Eucalyptol	—	—	—	74.87	—	—	1017	1037	RI, MS
4-Carene	2.48	—	—	—	—	—	1018	1014	RI, MS
β-Phellandrene	3.96	—	—	—	—	—	1026	1029	RI, MS
β-Terpinene	45.56	—	—	—	4.21	—	1044	1045	RI, MS
γ-Terpinene	3.23	—	—	0.34	—	—	1060	1065	RI, MS
neo-allo-Ocimene	—	—	—	—	3.02	—	1147	1144	RI, MS
Terpinen-4-ol	—	—	—	4.47	0.61	—	1162	1176	RI, MS
α-Terpineol	—	—	—	4.57	—	—	1181	1191	RI, MS, Co
Estragole	—	—	—	—	5.19	—	1195	1191	RI, MS
Piperitone	33.47	0.85	0.12	—	—	—	1227	1221	RI, MS
γ-Pyronene	—	—	4.23	—	—	21.97	1250	1252	RI, MS
2-Undecanone	—	—	—	—	1.92	—	1265	1274	RI, MS
Bornyl acetate	—	—	—	—	10.05	—	1272	1277	RI, MS
Safrole	—	—	38.08	—	—	—	1285	1281	RI, MS
α-Cubebene	—	6.75	—	—	12.05	—	1350	1359	RI, MS
α-Copaene	—	—	—	—	0.43	—	1372	1375	RI, MS
Isoledene	—	—	—	—	1.81	—	1376	1371	RI, MS
Geranyl acetate	—	—	—	—	1.66	—	1379	1386	RI, MS
Methyl eugenol	—	6.21	23.49	—	—	—	1384	1387	RI, MS
β-Elemene	—	4.89	0.18	0.41	11.44	1.02	1388	1398	RI, MS
β-Caryophyllene	0.51	26.17	4.00	—	7.16	7.77	1414	1418	RI, MS
α-Caryophyllene	—	3.78	0.28	1.15	2.48	4.65	1454	1453	RI, MS
γ-Muurolene	—	0.75	—	1.06	1.14	—	1465	1462	RI, MS
γ-Gurjunene	—	3.40	—	—	0.68	—	1474	1475	RI, MS
Germacrene D	—	—	0.84	—	5.73	20.98	1477	1479	RI, MS
2-Tridecanone	—	—	—	—	0.61	—	1481	1485	RI, MS
Valencene	—	0.79	—	—	—	—	1484	1481	RI, MS
α-Farnesene	—	—	—	—	—	14.28	1505	1508	RI, MS
Myristicin	—	6.75	28.74	—	—	—	1513	1510	RI, MS
δ-Cadinene	—	8.58	—	1.21	7.27	17.15	1520	1524	RI, MS
Spathulenol	—	10.19	—	0.36	1.85	4.42	1563	1561	RI, MS
Nerolidol	—	—	—	—	2.85	—	1566	1564	RI, MS
Caryophyllene oxide	—	13.36	—	—	—	4.32	1583	1585	RI, MS
Viridiflorol	—	6.50	—	—	—	—	1592	1595	RI, MS
τ-Cadinol	—	—	—	0.53	—	—	1640	1643	RI, MS
Monoterpenoids	93.62	0.85	42.43	90.88	26.13	21.97			
Sesquiterpenoids	4.47	85.16	5.30	4.19	54.89	74.59			
Others	—	12.96	52.23	—	14.24	—			
Total	98.09	98.97	99.96	95.07	95.26	96.56			

[a]RI, retention index of the chromatography determined on a HP-5MS column using the homologous series of *n*-hydrocarbons as reference; [b]RI, retention index reported in the literatures or libraries. [c]Identification method: RI, comparison of retention indices with published data; MS, comparison of mass spectra with those listed in the NIST 05 and Wiley 275 libraries and with published data; Co, coinjection with standard compound.

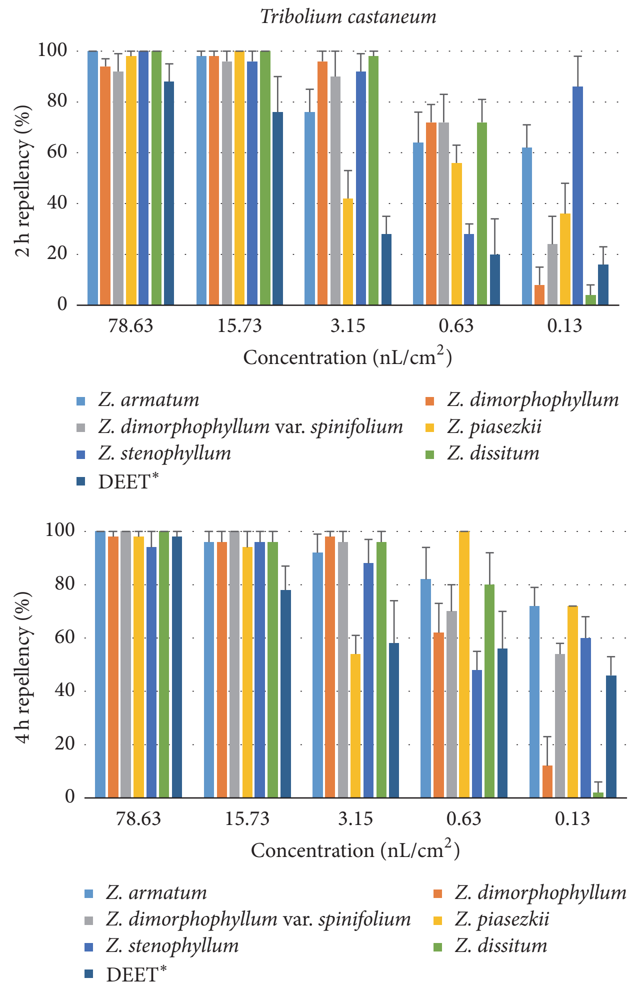

Figure 1: Percentage repellency (PR) of the essential oils from six *Zanthoxylum* species against *T. castaneum* at 2 and 4 h after exposure. *Data from Yang et al. [23].

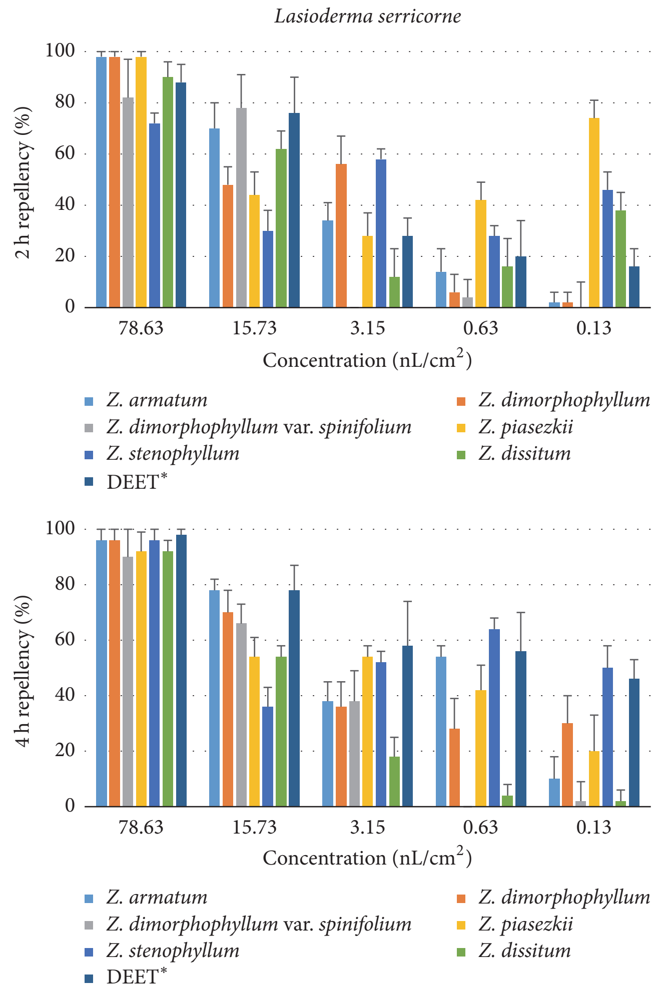

Figure 2: Percentage repellency (PR) of the essential oils from six *Zanthoxylum* species against *L. serricorne* at 2 and 4 h after exposure. *Data from Yang et al. [23].

possessed high repellent activity against *L. serricorne* adults only at the highest concentration of 78.63 nL/cm^2. Mostly, the repellent activity against *L. serricorne* adults decreased obviously when the concentration diluted. However, *Z. stenophyllum* essential oil showed higher repellent activity against *L. serricorne* than the positive control of DEET at lower concentrations of 3.15 and 0.63 nL/cm^2 at both 2 and 4 h after exposure. As secondary metabolites of natural plants, these essential oils with abundant resources have potential for the development as botanical repellents. The different repellent activities on two insects might be attributed to the different anti-insect mechanism and different nonpersistent volatility of essential oil samples. Since there are no sufficient reports about it at present, further investigations need to be conducted in the future.

4. Conclusions

In this study, we investigated the chemical composition of six essential oils from *Zanthoxylum* species; among them, the chemical constituents of essential oils from *Z. dimorphophyllum*, *Z. piasezkii*, and *Z. stenophyllum* were reported for the first time. What is more, repellent activities of these six essential oil samples against *T. castaneum* and *L. serricorne* adults were evaluated for the first time. It demonstrated that the essential oils of these six *Zanthoxylum* species essential oils possessed significant repellent activities against *T. castaneum* and *L. serricorne* adults. According to their abundant natural resources, these six essential oils of *Zanthoxylum* species with significant repellent activity might be developed into novel repellents to supply or substitute the heavy application of conventional repellents. Although further detailed investigations are needed, the above results can not only provide comprehensive utilization of plant resources of *Zanthoxylum* genus but also establish a very good perspective of novel application in control of stored-product insects.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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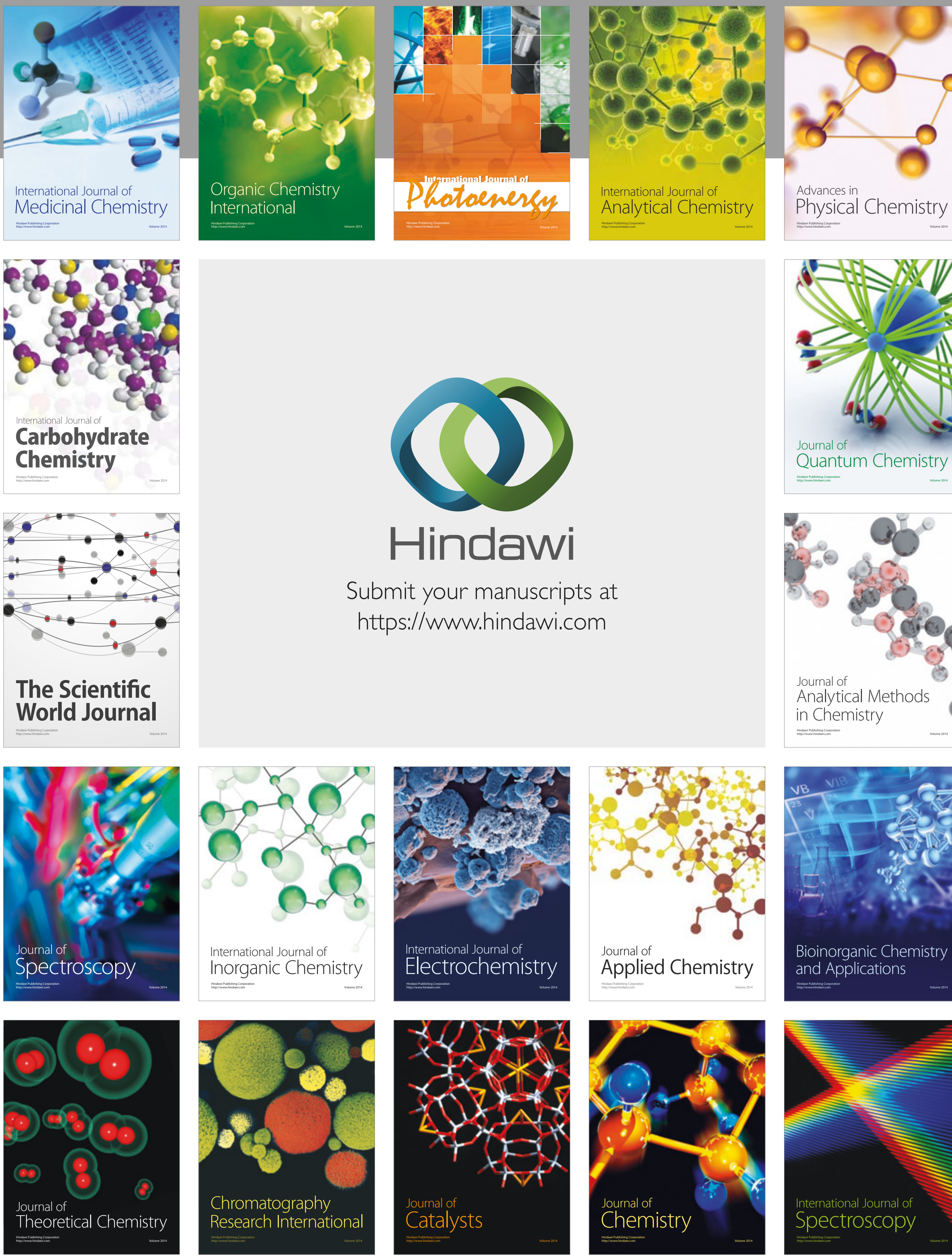
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