Peer-reviewed version available at *Molecules* **2016**, *21*, 1359; doi:10.3390/molecules21101359

Article

Chemical Compositions of *Ligusticum chuanxiong* Oil and Lemongrass Oil and Their Joint Action against *Aphis citricola* van der Goot (Hemiptera: Aphididae)

Chao Zhang [1], Runqiang Liu [1], Jun He [1], Zhiqing Ma [1,2,*] and Xing Zhang [1,2,*]

[1] Research and Development Center of Biorational Pesticide, Northwest Agriculture & Forestry University, Yangling 712100, China; zhangchao1990@nwsuaf.edu.cn (C.Z.); lrqiang1981@163.com (R.L.); hejun1963@yeah.net (J.H.)

[2] Shaanxi Research Center of Biopesticide Engineering & Technology, Yangling 712100, China

* Correspondence: zhiqingma@nwsuaf.edu.cn (Z.M.); zhxing1952@gmail.com (X.Z.); Tel.: +86-29-8709-2122 (Z.M.); +86-29-8709-2123 (X.Z.)

Abstract: In order to develop novel botanical insecticides, the joint action of *Ligusticum chuanxiong* oil (LCO) and lemongrass oil (LO) against *Aphis citricola* van der Goot was determined systematically indoors and outdoors. The chemical profiles of LCO and LO as determined by gas chromatography- mass spectrometry analysis revealed that main compounds from LCO were Z-Ligustilide (44.58%) and Senkyunolide A (26.92%), and that of LO were geranial (42.16%) and neral (32.58%), respectively. The mixture of LCO and LO showed significant synergy against *A. citricola*, with a common-toxicity coefficient (CTC) value of 221.46 at the optimal ratio of LCO to LO (4: 1, w/w). Based on the results of solvents and emulsifiers screening, *L. Chuanxiong* oil · Lemongrass oil 20% emulsifiable concentrate (20% LCO · LO EC) was developed, which was confirmed to meet the requirements of a commercial pesticide by quality test. Field trials indicated that the insecticidal activity of the diluted 20% LCO · LO EC (1000 fold dilution) was comparable to conventional pesticide (20% imidacloprid EC) on *A. citricola* 7 days after application. Thus, the mixture of LCO and LO has the potential to be further developed as a botanical pesticide.

Keywords: lemongrass oil; *Ligusticum chuanxiong* oil; *Aphis citricola* van der Goot; botanical aphicides

1. Introduction

Aphids (Hemiptera: Aphididae) are among the most destructive pests, widely classified in more than 4,300 described species [1]. Aphids threat crops by aphids through feeding on plants, transmitting plant pathogenic viruses, and secreting honeydew which could further lead to secondary fungal infection and inhibits photosynthesis [2-4]. Aphid has become one of the most serious pests in agriculture production because of rapid reproduction and specific feeding habits. Approximately, hundreds of millions dollars of crop losses are caused by aphids annually [5].

In the past few decades, various insecticides have been used to control the aphids [6]. As a result of frequent chemical application, aphids have developed high resistance to numerous commonly used insecticides in many agricultural areas. For example, the green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), has developed resistance to at least seventy different synthetic compounds [7], and cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is resistant to carbamate, organophosphorus (OP) pyrethroid and neonicotinoids insecticides [8,9].

Certain plant essential oils are widely used in the flavoring and fragrance industries and in aromatherapy. They can be obtained by expression, fermentation, enfleurage or extraction, while steam distillation is most commonly used in commercial. Moreover, some aromatic plants have traditionally been used to protect stored products, but the development potential of plant essential oils for broad-spectrum pest management has only been realized recently [10]. Both antimicrobial efficacy and insecticidal effects of the essential oils have been reported [11-13]. Recent investigations

in several countries confirmed that some plant essential oils not only repel insects, but also have contact and fumigant activities against specific pests, and fungicidal actions against some important plant pathogens [14]. Essential oils of cumin (*Cuminum cyminum* L.), anise (*Pimpinella ansium* L.), oregano (*Origanum syriacum* L.) and eucalyptus (*Eucalyptus dives*) were confirmed to be effective as fumigants against two greenhouse pests, the cotton aphid (*Aphis gossypii*) and carmine spider mite (*Tetranychus cinnabarinus*)[15].

Ligusticum chuanxiong Hort. (family Umbeliferae) is also known as Chinese lovage, which has been employed as a traditional Chinese medicine in folk remedies for long [16]. Twenty compounds have been identified in the essential oil from L. chuanxiong, and the major compounds are phenolics [17]. As a consequence, it is widely applied in food preparation as an antioxidant [16]. Moreover,. *L. chuanxiong* oils (LCO) have been found to possess insecticidal activity against maize weevils, *Sitophilus zeamais* (Coleoptera: Curculionidae) [18].

Cymbopogon citratus is one of the most commonly used plants for the treatment of nervous and gastrointestinal disturbances and the antibacterial properties of its essential oil have been studied [19]. Lemongrass oil (LO), an important oil, has been shown to reduce aflatoxin formation and impede fungal growth of *Aspergillus flavus* Link. in stored rice [19,20]. The quality of LO is generally determined by its citral content, and citral (3,7-dimethyl-2,6-octadienal) consists of cis-isomer geranial and the trans-isomer neral [21,22].

However, few researches in field trials and formulation preparations of plant essential oils for aphid management were reported. The objective of this study was to assess in more details the potential of LCO and LO to control *Aphis citricola* (Hemiptera: Aphididae). This study is mainly focused on the bioactivities and the synergistic effect of LCO and LO against *A. citricola* indoors and outdoors. A LCO · LO 20% emulsifiable concentrate (LCO · LO 20% EC) was successfully used in field trials with a significant effect against *A. citricola*.

2. Results

2.1. Toxicity of LCO and LO against Aphis citricola

Bioassays of 20% LCO EC and 20% LO EC against *A.citricola* were conducted and the results were shown in Table 1. Both LCO and LO showed high toxicity against *A.citricola*, with LC_{50} values of 128.8 and 169.6 mg/L respectively after twenty-four hours treatment.

Table 1. Toxicity of *Ligusticum chuanxiong* oil (LCO) and Lemongrass oil (LO) against *Aphis citricola* van der Goot (24h)

Pesticide	LC-P（Y=）	LC_{50}（mg/L）	r	Confidence interval of LC_{50} (P <0.05)	χ^2
20% LCO EC	-5.55+4.99x	128.78	0.9822	116.44～142.42	5.45
20% LO EC	-6.56+5.18x	169.59	0.9922	162.75～169.71	3.71

LC_{50} value was determined by log-probit analysis.

2.2. Identification and quantification of compounds from LCO and LO

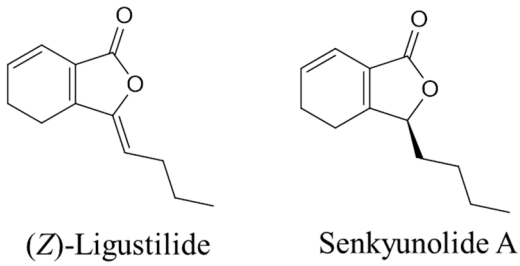
The list of principal identified compounds from LCO and LO is given in Table 2. By comparing the mass spectra data of the sample with literature data, eight main compounds of LCO were identified as *(Z)*-ligustilide (44.58%), senkyunolide A (26.92%), neocnidilide (6.21%), 3-n-butylphthalide (4.86%), butylidenephthalide (2.95%), β-selinene (2.15%), 1,3,5-undecatriene (1.83%) and *E*-ligustilide (1.56%). Meanwhile, eight main compounds of LO were identified as

Peer-reviewed version available at *Molecules* **2016**, *21*, 1359; doi:10.3390/molecules21101359

(*Z*)-geranial (42.16%), (*E*)-neral (32.58%), β-myrcene (4.95%), geranyl acetate (4.28%), terpinolene (1.58%), isopulegol (1.38%), trans-bergamotene (0.85%) and citronellal (0.38%).

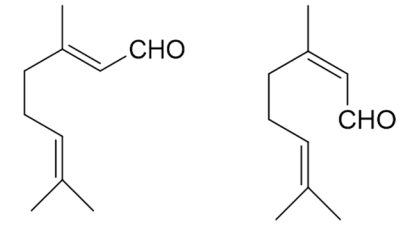
Table 2. Identification and Quantification of Principal Compounds from *Ligusticum Chuanxiong* Oil and Lemongrass Oil in GC-MS

***Ligusticum Chuanxiong* Oil**		**Lemongrass Oil**	
Compound	**Relative Content (%)**	**Compound**	**Relative Content (%)**
Z-Ligustilide	44.58	Geranial	42.16
Senkyunolide A	26.92	Neral	32.58
Neocnidilide	6.21	*β*-Myrcene	4.95
3-*n*-Butylphthalide	4.86	Geranyl acetate	4.28
Butylidenephthalide	2.95	Terpinolene	1.58
β-Selinene	2.15	Isopulegol	1.38
1,3,5-Undecatriene	1.83	*trans*-Bergamotene	0.85
E-Ligustilide	1.56	Citronellal	0.38



(*Z*)-Ligustilide Senkyunolide A

Figure. 1 Structures of (*Z*)-Ligustilide and Senkyunolide A.



(*Z*)-Geranial (*E*)-Neral

Figure. 2 Structures of (Z)-Geranial and (*E*)-Neral.

2.3. Cooperative virulence index (c.f) of LCO and LO

Bioassay of the mixture of LCO and LO was carried out and the c.f value of the mixtures were determined. Data in Table 3 indicated that the mixture of LCO and LO has a synergistic effect, with a c.f value of 23.17.

Table 3. Cooperative virulence index (c.f) of mixtures combined by *Ligusticum chuanxiong* oil (LCO) and Lemongrass oil (LO) against *Aphis citricola* van der Goot (24 h)

Combinations	**Dilution times**	**Theoretical mortality (%)**	**Actual mortality (%)**	**c.f**
LCO/LO	1500	67.08	82.62	23.1727

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2016 doi:10.20944/preprints201609.0021.v1

Peer-reviewed version available at Molecules 2016, 21, 1359; doi:10.3390/molecules21101359

2.4. Confirmation of the best proportion of the mixture

On the basis of the results above, a series of tests were carried out to confirm the optimal proportion of LCO and LO. Table 4 shows that when the LC50 ratios LCO: LO were 80:20 and 90:10 (3.06:1 and 6.88:1 (w:w), respectively), relative high poison ratios of 1.22 and 1.21 was obtained. According to the characteristic of EC preparations, the optimum efficient and effective ratio of *L. chuanxiong* oil and lemongrass oil was established at 4:1 (w:w).

Table 4. Toxicity of mixtures with different LC50 ratio of *Ligusticum chuanxiong* oil (LCO) and lemongrass oil (LO) against *Aphis citricola* van der Goot (24 h)

LCO LC_{50}%	100	90	80	70	60	50	40	30	20	10	0
LO LC_{50} %	0	10	20	30	40	50	60	70	80	90	100
Actual mortality(%)	52.11	62.5	62.62	56.31	51.94	54.76	49.46	51.09	48.02	48.5	48.02
Theoretical mortality(%)	52.11	51.70	51.29	50.88	50.47	50.07	49.66	49.25	48.84	48.43	48.02
Poison ratio	1	1.21	1.22	1.11	1.03	1.09	1.00	1.04	0.98	1.00	1

LC_{50} values of LCO and LO were 128.78 and 169.59 mg/L, respectively. In this study, the LC_{50} values of LCO and LO were set at 130 mg/L and 170 mg/L, respectively.

2.5. CTC value of L. chuanxiong oil and lemongrass oil

According to the results above, 20% LCO · LO EC (containing 16% LCO and 4% LO) was designed and prepared. Bioassays of 20% LCO EC, 20% LO EC and 20% LCO·LO EC against A. citricola were conducted. Table 5 shows that compared with LCO and LO, the control effect of 20% LCO · LO is more significant with a LC_{50} of 61.09 mg/L, which indicates that this formula has a synergistic effect, with a CTC value >180.

Table 5. Co-toxicity coefficient (CTC) value of 20% LCO·LO EC to *Aphis citricola* van der Goot

Pesticide	**LC-P (Y=)**	**LC_{50}(mg/L)**	**Confidence interval of LC_{50} (*P* <0.05)**	**r**	***χ2***	**CTC**
20% LCO EC	-5.55+4.99x	128.78	116.44～142.42	0.9822	5.45	-
20% LO EC	-6.56+5.19x	169.59	162.75～169.71	0.9922	3.71	-
20%LCO·LO EC	7.42+1.99 x	61.09	51.56～72.36	0.9768	2.03	221.46

LC_{50} value was determined by log-probit analysis.

3.6. Preparation and quality test of 20% LCO · LO EC

Formulation of 20% LCO · LO EC was confirmed after the screening of solvents and emulsifiers. Then quality tests and bioassay were conducted according to the GB/T1603-79(89) standard. The formulation was a single-phase transparent liquid (pH 6.46), and there was no floating oil or sediment, which satisfied emulsification level II. After cold (0 °C) and thermal storage (54 °C) for 7 days and 12 days respectively, the preparation remained single-phase transparent liquid. From Table 6, relative high activity of 20% LCO · LO EC against *A. citricola* after the thermal storage treatment was obtained. And a significant synergy with a CTC value > 200 was still determined. Thus confirmed that thermal storage stability of 20% LCO · LO EC was excellent.

Table 6. Toxicity of 20% LCO·LO EC before and after hot storage to *Aphis citricola* van der Goot (24 h)

Pesticide	LC-P（Y=）	LC_{50}(mg/L)	r	Confidence interval of LC_{50} (P <0.05)	χ^2	CTC value
20% LCO·LO EC After heating storage test	0.62+2.42x	64.32	0.9784	55.16～75.01	2.67	210.34

LC_{50} value was determined by log-probit analysis.

3.7. Field trials of 20% LCO · LO EC against Aphis citricola

Field trial of 20% LCO · LO EC against *A. citricola* was carried out in Baishui county (Table 7) and Yangling city (Table 8), Shaanxi province. Tables 7 and 8 indicate that 20% LCO · LO EC exhibited significant control of *A. citricola*. The control effects of 500 times dilution were 90.06% (Table 7) and 87.24% (Table 8) which were comparable to 1000 times dilution of 20% imidacloprid EC. Meanwhile, the control at 1000 times dilution of 20% LCO · LO EC was still more than 80% seven days after the treatment.

Table 7. The result of field trials of 20% LCO·LO EC on *Aphis citricola* van der Goot (October, 2008, Baishui, Shaanxi province)

Pesticide	Dilution	Corrected efficacy（%）		
		1 day after trial	3 days after trial	7 days after trial
20%LCO·LO EC	500	46.08 ± 5.01 b	60.34 ± 4.32 b	90.06 ± 3.23 b
	1000	43.99 ± 3.95 b	58.87 ± 3.08 b	80.76 ± 5.28 c
	1500	37.45 ± 4.06 b	55.84 ± 5.36 b	63.88 ± 4.95 d
20% Imidacloprid EC	1000	99.04 ± 5.36 a	100.0 ± 0 a	100.0 ± 0 a

Data are the mean of three replicates (50 aphids per replicate) and are represented as mean±standard deviation. Means in the same column followed by the same lower case letter are not significantly different (P<0.05) in a Tukey test.

Table 8. The result of field trials of 20% LCO·LO EC on Aphis citricola van der Goot (June, 2012, Yangling, Shaanxi province)

Pesticide	Dilution	Corrected efficacy（%）		
		1 day after trial	3 days after trial	7 days after trial
20% LCO·LO EC	500	50.28 ± 5.63 b	63.31 ± 5.39 b	87.24 ± 1.62 b
	1000	47.67 ± 4.16 b	60.56 ± 4.87 b	80.69 ± 2.31 c
	1500	32.56 ± 4.01 c	49.23 ± 3.18 c	60.47 ± 4.28 d
20% Imidacloprid EC	1000	97.76 ± 1.05 a	100.0 ± 0 a	100.0 ± 0 a

Data are the mean of three replicates (50 aphids per replicate) and are represented as mean±standard deviation. Means in the same column followed by the same lower case letter are not significantly different (P<0.05) in a Tukey test.

3. Discussion

Plant essential oil insecticides have been accepted for their high bioactivities and specific modes of action [10]. Recent investigations indicate that some chemical constituents of essential oils interfere with the octopaminergic nervous system insects[23]. As this target site is not shared with mammals, these essential oil chemicals are relatively non-toxic to mammals and fish in toxicological tests, and meet the criteria for “reduced risk” pesticides. This special regulatory status combined with the wide availability of essential oils in the flavor and fragrance industries has made it possible to fast-track commercialization of essential oil-based pesticides in USA [24]. It has been shown that

L. chuanxiong oil and lemongrass oil are promising as pesticides and activities against various kinds of pests [10,25-27]. However, there are few studies focused on the combined activities of these two oils against aphids.

This study has confirmed that both LCO and LO have significant bioactivity against *Aphis citricola* with LC_{50} values of 128.8 mg/L and 169.6 mg/L, respectively. A mixture of these two essential oils shows a synergistic effect. Based on the field trials, the 20% LCO · CO EC preparation could be used as an alternative to imidacloprid. These two essential oils have promising potential to be developed into novel biorational pesticides. However, only one species of aphid was tested in this study, and a wider control spectrum needs to be confirmed in field trials.

The need for chemical standardization and quality control is still a main barrier for the commercialization of essential oil-based pesticides [28,29]. Table 6 confirms the synergistic action that all parameters of LCO · LO EC and the heat stability of the active ingredient act against the target insect in the LCO · LO EC formulation. Botanical pesticides, especially plant essential oils, would be advantageous in terms of pest resistance and behavior desensitization as they are the mixtures of natural mixtures, acting synergistically against pest insects, which would be advantageous in terms of pest resistance and behavior desensitization [28,30]. However, quality control is an urgent issue for botanical pesticide registration [27]. In this study, the stability of bioactive principle in botanical pesticides is another important parameter for botanical pesticide registration.

In conclusion, our study showed that LCO and LO displayed significant bioactivities against. *A. citricola*. Based on this, we have developed an essential oil-based pesticide that effectively controls *A. citricola* in the field. Thus, these two oils are worthy of development as novel biorational pesticides.

4. Materials and Methods

4.1 Materials

Aphis. citricola were collected from apple trees at the Horticulture College of Northwest A&F University.

LCO and LO were purchased from Guangzhou Hengxin Flv. & Frag. Co., LTD, and a 20% emulsifiable concentrate formulation (EC) prepared for this investigation.

20% Imidacloprid EC was purchased from Henan Planck bio-chemical industry co., LTD, China.

All the accessory ingredients, solvents and other reagents were industrial products, which were purchased from Aladdin Industrial Corporation, China.

4.2 Bioassay

Essential oils were dissolved in acetone to a concentration of 100 mg/mL, and then diluted with 0.05% Tween 80 into stock solutions at a concentration of 10 mg/mL. Working solutions were diluted from stock solutions with 0.05% Tween 80. Controls were 0.05% Tween 80 solutions with acetone at the same concentration as the treatment solutions. Clean apple leaves were collected and cut into leaf discs (each leaf disc was containing about 15~20 aphids). Each disc (3.5 cm diameter) was dipped into the working solutions of essential oils for 10s with each leaf disc receiving >100 μL of the solution. The discs were then air-dried in glass Petri dishes (9 cm diameter) at room temperature. After the treatment, each leaf disc was placed individually in a covered glass Petri dish, and then incubated at temperature 25°C with 14:10 h light:dark. Mortality was counted after 24h with three replicates for each treatment.

4.3 Gas Chromatography-Mass Spectrometry (GC/MS) analysis

The two essential oils, LCO and LO were analyzed by a capillary GC/MS (Model GCMS-QP2010, Shimadzu, Kyoto, Japan). The gas chromatographic conditions were as followed: GC oven fitted with a DB-5 MS 30 m capillary column (0.25 mm I.D., 0.25 μm film, thickness, Aglient, Palo Alto, CA, USA) with carrier gas helium at flow rate of 1.2 mL/min, operating under an

initial temperature at 80 °C for 2 min up to 250 °C in ramp rate of 10 °C/min, then held for 11min. 1.0 μL sample solution was injected into the system with a split ratio of 1:50 for analysis. The electron impact ionization mass spectrometer was operated with an ionization voltage of 70eV and an ion source temperature at 200 °C, using a scan mode and measuring total ions chromatogram (TIC) under a mass range of 50.0-350.0. The datum analysis was performed on a NIST library [31,32] (Shimadzu, Kyoto, Japan).

4.4 Joint action of the two essential oils

Mixture of LCO and LO at their respective LC_{50} (128.78 and 169.59 mg/L) was prepared, and the bioactivity against *Aphis. citricola* was determined using the above mentioned bioassay method as well as the joint action of the two essential oils were measured according to Mansour's method [33]. When cooperative virulence index (c.f) of the mixture is > 20, the mixture showed a synergistic effect. Similarly, when the c.f < -20, an antagonistic effect was shown. In the range of c.f between -20 and 20, there was only an additive effect.

4.5 Determination of efficiency ratio and the synergistic effect

Following Yi's method [34], serial mixtures were prepared at a LC50 ratio (LCO/LO) of 10/0, 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 9/1 or 0/10. The best ratio of the two oils and the virulence regression lines and median lethal concentrations (LC50) of monomers and mixtures were obtained. Co-toxicity coefficient (CTC) values of the mixtures were calculated by the method of Sun [35]: with CTC > 120 defined as a synergistic effect, CTC < 80 as an antagonistic effect, while any CTC values fallen into the range of 80 to120, indicates additive effects. The ratio of 4:1 (LCO:LO, w:w) was selected for the 20% LCO · LO EC formulation.

4.6 Studies of EC Formulations of 20% LCO · LO

Optimum ratios of oil and surfactant were determined according to Wiwattanapatapee's method [28]. The most suitable formulation that gave a clear homogeneous liquid with stable emulsion after 1:20 dilution in water were obtained and the ones with the best characteristics were selected for the preparation of the LCO · LO 20% EC.

The LCO · LO 20% EC was prepared by simple mixing [36]. The oil mixture (16g LCO and 4g LO) was mixed with pesticide emulsifier 1601 (10mL) using a mortar and pestle to obtain a homogeneous concentrate mixture, and then brought the volume up to 100mL by ethyl acetate. Thus 20% LCO · LO EC (w/v) was prepared. The formulation was stored at room temperature (25±3 °C) and protected from light.

Evaluation of the physical properties of 20% LCO · LO EC was processed according to Wiwattanapatapee's method [36]. EC (2.5g) was dispersed in 50 mL distilled water by stirring with a magnetic stirrer at 500 rpm for 5min at room temperature (25±3 °C) for emulsification. The pH of the emulsion was also determined with a pH-meter (Mettler-Toledo Co. Ltd.).

According to the GB/T1603-79(89) standard, the stabilities of the emulsion after cold storage (0 °C, 7 days) and thermal storage (54 °C, 12 days) were determined, respectively.

4.7 Field Trials of the Preparations

Field trials of the preparation against *A. citricola* were conducted in Bai Shui county (October, 2008) and Yangling city (June, 2012), Shaanxi province, respectively. The prepared EC formulation was diluted 500, 800 and 1000 times, and then applied by constant spraying method (spray volume of 600 L/hm^2). In addition, 20% imidacloprid EC (1,000 times dilution) was applied as a positive control. Water alone was applied as the negative control. The plots were arrayed randomly. Each treatment was replicated three times. Five to ten leaves on fixed plants were marked and numbers of

aphids on the leaves were recorded (over 200 aphids in every replicate). Numbers of aphids on the marked leaves on the 1st, 3rd and 7th days after spraying were counted and the control effect calculated according to Abbott's formula [37]. Corrected control effect was transformed against the negative control then processed to statistical analysis using the SPSS software.

4.8 Statistical analysis

Percent mortality was calculated using the ratio of dead insects to the total number of insects after 24 hours, and mortality data were corrected using Abbott's formula. Probit analysis was used to calculate LC_{50} (concentration causing 50% mortality compared with the control) values and their confidence intervals [38]. Statistical analysis in this study was processed with SPSS 19.0 one-way ANOVA to assess the significance of difference between groups. The acceptance level of significance was $P < 0.05$. The results were expressed as mean ± S.D.

Acknowledgments:

We are thankful to the reviewers for helpful comments. The study has been supported by the Special Fund for Agro-scientific Research in the Public Interest of the People's Republic of China (No. 200903052).

Author Contributions:

Z.M. and X.Z. conceived and designed the experiments; C.Z. and R.L. performed the experiments; C.Z. and Z.M. analyzed the data; J.H. and X.Z. contributed reagents/materials/analysis tools; C.Z. and Z.M. wrote the paper.

Conflicts of Interest:

The authors declare no conflict of interest.

References

1. Dedryver, C.-A.; Le Ralec, A.; Fabre, F. The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *CR. Bio* **2010**, *333*, 539-553.
2. Edwards, O.R.; Franzmann, B.; Thackray, D.; Micic, S. Insecticide resistance and implications for future aphid management in australian grains and pastures: A review. *Anim. Prod. Sci* **2008**, *48*, 1523-1530.
3. Foster, S.; Denholm, I.; Devonshire, A. The ups and downs of insecticide resistance in peach-potato aphids (myzus persicae) in the uk. *Crop Prot* **2000**, *19*, 873-879.
4. Strange, R.N.; Scott, P.R. Plant disease: A threat to global food security. *Phytopathology* **2005**, *43*.
5. Hagenbucher, S.; Wäckers, F.L.; Romeis, J. Aphid honeydew quality as a food source for parasitoids is maintained in bt cotton. *PloS One* **2014**, *9*, e107806.
6. Yang, S.; Fitches, E.; Pyati, P.; Gatehouse, J.A. Effect of insecticidal fusion proteins containing spider toxins targeting sodium and calcium ion channels on pyrethroid‐resistant strains of peach‐potato aphid (*Myzus persicae*). *Pest. Manag. Sci* **2015**, *71*, 951-956.
7. Silva, A.X.; Jander, G.; Samaniego, H.; Ramsey, J.S.; Figueroa, C.C. Insecticide resistance mechanisms in the green peach aphid *Myzus persicae* (hemiptera: Aphididae) i: A transcriptomic survey. *PLoS One* **2012**, *7*, e36366.
8. Moores, G.D.; Gao, X.; Denholm, I.; Devonshire, A.L. Characterisation of insensitive acetylcholinesterase in insecticide-resistant cotton aphids, *Aphis gossypiiglover* (homoptera: Aphididae). *Pestic. Biochem. Phys* **1996**, *56*, 102-110.
9. Gore, J.; Cook, D.; Catchot, A.; Leonard, B.; Stewart, S.; Lorenz, G.; Kerns, D. Cotton aphid (heteroptera: Aphididae) susceptibility to commercial and experimental insecticides in the southern united states. *J. Econ. Entomol* **2013**, *106*, 1430-1439.

10. Isman, M.B.; Miresmailli, S.; Machial, C. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochem. Rev* **2011**, *10*, 197-204.
11. Gutierrez, J.; Barry-Ryan, C.; Bourke, P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *Int. J. Food. Microbiol* **2008**, *124*, 91-97.
12. Daferera, D.J.; Ziogas, B.N.; Polissiou, M.G. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, fusarium sp. And clavibacter michiganensis subsp. Michiganensis. *Crop. Prot* **2003**, *22*, 39-44.
13. Konstantopoulou, I.; Vassilopoulou, L.; Mavragani-Tsipidou, P.; Scouras, Z. Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven greek aromatic plants on *Drosophila auraria*. *Experientia* **1992**, *48*, 616-619.
14. Isman, M.B. Plant essential oils for pest and disease management. *Crop. Prot* **2000**, *19*, 603-608.
15. Tunc, I.; Şahinkaya, Ş. Sensitivity of two greenhouse pests to vapours of essential oils. *Entomol. Exp. Appl* **1998**, *86*, 183-187.
16. Yuan, J.F.; Zhang, Z.Q.; Fan, Z.C.; Yang, J.X. Antioxidant effects and cytotoxicity of three purified polysaccharides from *Ligusticum chuanxiong* hort. *Carbohyd. Polym* **2008**, *74*, 822-827.
17. Jeong, J.B.; Ju, S.Y.; Park, J.H.; Lee, J.R.; Yun, K.W.; Kwon, S.T.; Lim, J.-H.; Chung, G.Y.; Jeong, H.J. Antioxidant activity in essential oils of *Cnidium officinale* makino and *Ligusticum chuanxiong* hort and their inhibitory effects on DNA damage and apoptosis induced by ultraviolet b in mammalian cell. *Cancer. Epidemiol* **2009**, *33*, 41-46.
18. Chu, S.S.; Jiang, G.H.; Liu, Z.L. Insecticidal components from the essential oil of chinese medicinal herb, *Ligusticum chuanxiong* hort. *J. Chem* **2011**, *8*, 300-304.
19. Paranagama, P.; Abeysekera, K.; Abeywickrama, K.; Nugaliyadde, L. Fungicidal and anti - aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (dc.) Stapf.(lemongrass) against *Aspergillus flavus* link. Isolated from stored rice. *Lett. App. Microbiol* **2003**, *37*, 86-90.
20. Tzortzakis, N.G.; Economakis, C.D. Antifungal activity of lemongrass (*Cympopogon citratus* l.) essential oil against key postharvest pathogens. *Innov. Food. Sci. Emerg* **2007**, *8*, 253-258.
21. Olivero - Verbel, J.; Nerio, L.S.; Stashenko, E.E. Bioactivity against *Tribolium castaneum* herbst (coleoptera: Tenebrionidae) of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils grown in colombia. *Pest. Manag Sci* **2010**, *66*, 664-668.
22. Enan, E. Insecticidal activity of essential oils: Octopaminergic sites of action. *Comp. Biochem Phys C* **2001**, *130*, 325-337.
23. Koul, O.; Walia, S.; Dhaliwal, G. Essential oils as green pesticides: Potential and constraints. *Biopestic. Inter* **2008**, *4*, 63-84.
24. Huang, Y.; Pu, F. Studies on the chemical components of the essential oil from the rhizome of *Ligusticum sinense* oliv.C.V. Chuanxiong hort. *Acta Pharmaceutica Sinica* **1988**, *6*, 005.
25. Kwon, J.-H.; Ahn, Y.-J. Acaricidal activity of butylidenephthalide identified in *Cnidium officinale* rhizome against dermatophagoides farinae and dermatophagoides pteronyssinus (*Acari: Pyroglyphidae*). *J. Agr. Food. Chem* **2002**, *50*, 4479-4483.
26. Sharaby, A. Anti-insect properties of the essential oil of lemon grass, *Cymbopogen citratus* against the lesser cotton leafworm *Spodoptera exigua* (hbn). *Insect Sci Applic* **1988**, *9*, 465-468.
27. Isman, M.B. Neem and other botanical insecticides: Barriers to commercialization. *Phytoparasitica* **1997**, *25*, 339-344.

28. Isman, M.; Regnault-Roger, C.; Philogène, B.; Vincent, C. Problems and opportunities for the commercialization of botanical insecticides. *Biopestic. Plant. ori* **2005**, 283-291.
29. Khater, H.F. Prospects of botanical biopesticides in insect pest management. *Pharmacologia* **2012**, *3*, 641-656.
30. Boukhatem, M.N.; Ferhat, M.A.; Kameli, A.; Saidi, F.; Kebir, H.T. Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs. *Libyan. J. Med* **2014**, *9*, 58-62.
31. Zhang, X.; Xiao, H.; Xu, Q.; Li, X.; Wang, J.; Liang, X. Characterization of phthalides in *Ligusticum chuanxiong* by liquid chromatographic-atmospheric pressure chemical ionization-mass spectrometry. *J. Chromatogr Sci* **2003**, *41*, 428-433.
32. Li, H. X.; Ding, M. Y.; Yu, J. Y. Separation and identification of the phthalic anhydride derivatives of *Liqusticum chuanxiong* hort by gc-ms, tlc, hplc-dad, and hplc-ms. *J. Chromatogr Sci* **2002**, *40*, 156-161.
33. Mansour, N.; Eldefrawi, M.; Toppozada, A.; Zeid, M. Toxicological studies on the egyptian cotton leaf worm, *Prodenia litura*. Vi. Potentiation and antagonism of organophosphorus and carbamate insecticides. *J. Econ. Entomol* **1966**, *59*, 307-311.
34. Yi, F.; Zou, C.; Hu, Q.; Hu, M. The joint action of destruxins and botanical insecticides (rotenone, azadirachtin and paeonolum) against the cotton aphid, *Aphis gossypii* glover. *Molecules* **2012**, *17*, 7533-7542.
35. Sun, Y.-P.; Johnson, E. Analysis of joint action of insecticides against house flies. *J. Econ. Entomol* **1960**, *53*, 887-892.
36. Wiwattanapatapee, R.; Sae-Yun, A.; Petcharat, J.; Ovatlarnporn, C.; Itharat, A. Development and evaluation of granule and emulsifiable concentrate formulations containing *Derris elliptica* extract for crop pest control. *J. Agr. Food. Chem* **2009**, *57*, 11234-11241.
37. Abbott, W. A method of computing the effectiveness of an insecticide. *J. econ. Entomol* **1925**, *18*, 265-267.
38. Finney, D.J. Probit analysis. *J. Inst. Actuaries* **1952**, *78*, 388-390.