

#### 한국응용곤충학회지

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# Insecticidal Activity of Cinnamon Essential Oils, Constituents, and (*E*)- Cinnamaldehyde Analogues against *Metcalfa pruinosa* Say (Hemiptera: Flatidae) Nymphs and Adults

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### 미국선녀벌레(Metcalfa pruinosa Say)에 대한 계피 정유 유래 물질의 실충 활성

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**ABSTRACT:** The insecticidal activity of the constituents of cinnamon essential oils and structurally related compounds against both the nymphs and adults of the citrus flatid planthopper *Metcalfa pruinosa* was examined using a direct-contact application. The toxicity of the cinnamon oil constituents and 21 (*E*)-cinnamaldehyde related compounds regarding the nymphs of *M. pruinosa* was evaluated using a leaf-dipping bioassay. Based on 24 h LC<sub>50</sub> values, hydro-cinnamic acid (1.55 mg/cm<sup>2</sup>) is the most toxic compound, followed by geranic acid (1.59 mg/cm<sup>2</sup>). The LC<sub>50</sub> values of 11 of the compounds including cinnamaldehyde are between 1.60 mg/cm<sup>2</sup> and 4.94 mg/cm<sup>2</sup>. Low toxicities and no toxicity were observed with the other 15 (5.24 mg/cm<sup>2</sup> to 13.47 mg/cm<sup>2</sup>) and two compounds, respectively. Also, the toxicities of the cinnamon oil constituents and 21 cinnamaldehyde related compounds regarding the *M. pruinosa* adults were evaluated using a direct-spray method. The toxicity of eugenol (10.81 mg) is the most toxic compound for the adults of *M. pruinosa*, followed by geranic acid (30.68 mg). The LC<sub>50</sub> values of nine of the compounds including cinnamaldehyde are between 59.16 mg and 96.70 mg. Low toxicities and no toxicity were observed with the other 15 (105.44 mg to 255.76 mg) and three compounds, respectively. The spray formulations that comprise cinnamon bark and cinnamon green leaf oils resulted in 82.3% and 82.9% mortalities, respectively, toward the *M. pruinosa* adults in a ginseng field. Global efforts to reduce the level of highly toxic synthetic insecticides in agricultural environments justify further studies on cinnamon oils to ascertain whether the corresponding active principles can act as insecticides, when they are applied as a direct spray with contact action, for the control of *M. pruinosa* populations.

Key words: Metcalfa pruinosa, Cinnamon essential oils, Cinnamaldehyde, Spray formulation

조록: 본 연구에서는 계피 정유 3종의 구성성분을 분석하였고, 미국선녀벌레에 대한 살충활성을 검정하였다. (E)-Cinnamaldehyde을 포함한 9종의 계피 정유 구성성분과 21종의 유사 물질을 미국선녀벌레 약충에 대해 살충활성을 검정한 결과, hydro-cinnamic acid가 반수치사농도 1.55 mg/cm²로 가장 좋은 살충활성을 보였으며, geranic acid도 1.59 mg/cm²로 높은 살충 활성을 보였다. Cinnamaldehyde를 포함한 hydro-cinnamaldehyde, (E)-cinnamaldehyde, cinnamaldehyde, cinnamaldehyde, cinnamaldehyde, cinnamaldehyde, cinnamaldehyde, bonyl acetate 12종이 중간정도의 활성(1.60 - 4.94 mg/cm²)을 보였으며, 다른 물질들은 살충활성이 낮거나 없었다. 미국선녀벌레 성충에 대해서는 eugenol 이 반수치사 농도 10.81 mg로 가장 높은 살충활성을 보였으며, geranic acid (30.68 mg)도 높은 살충력을 보였다. Cinnamaldehyde 등 9종이 반수 치사 농도 105.44~255.76 mg의 살충활성을 보였다. 다른 18종의 물질은 활성이 낮거나 없었다. 실제 포장인 인삼포장에 발생하는 미국선녀벌레에 대한 적용시험에서 cinnamon bark 정유와 cinnamon green leaf 정유가 각각 82.3%와 82.9%의 높은 살충활성을 보였다. 농업환경에서 고독성 합성살충제의 사용을 줄일 수 있는 방안으로 본 논문에서 선발한 계피정유가 미국선녀벌레의 약충 및 성충 방제에 유용한 수단으로 이용될 수 있을 것으로 사료된다.

검색어: 미국선녀벌레, 계피정유, Cinnamaldehyde, 분무제형

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In recent years, due to the active introduction of foreign planting stock, and probably under the influence of currently changing climatic conditions, the number of invasive insect species in the Korean territory has been steadily increasing. The citrus flatid planthopper *Metcalfa pruinosa* (Hemiptera: Flatidae) (Say, 1830) is a North American species that was accidentally introduced into Italy in 1979 (Zangheri & Donadini, 1980). It belongs to the Flatidae family that is one of the largest groups of Fulgoroidea (Hemiptera: Auchenorrhyncha) (Nault & Rodriguez, 1985). Since the species was found in Seoul in 2009, it has also been identified in orchards in the Chungbuk and Kyungnam provinces of South Korea (Kim et al., 2011).

The citrus flatid planthopper is a broad polyphage damaging over 200 plant species, including field crops and ornamental plants (Mead, 1969). This planthopper is polyphagous and feeds on a wide variety of plants throughout the Mediterranean (Pons et al., 2002; Souliotis et al., 2008).

For the purpose of management, the presence of the dryinid wasp parasite *Psilodryinus typhlocybae* (Ashmead) has been reported as a common occurrence on the nymphs of the citrus flatid planthopper and its relatives. Chemical control measures against dense nymph and adult populations might be justifiable when they are applied to valuable trees; however, increasing public concerns regarding the environmental effects of insecticides including groundwater contamination, human-health harms, insecticidal residues on host plants, and the undesirable effects on non-target organisms intensify when continued or repeated applications of conventional insecticides become necessary.

There is therefore an urgent need for the development of effective management alternatives that do not harm environments and non-target organisms. Plant-derived insecticide have been suggested as potential alternatives to conventional insecticide, largely because plants and plant essential oils constitute a potential source of bioactive secondary metabolites that pose fewer risks to the environment and result in minimal impacts on human health (Ahn et al., 2006; Isman, 2006); in fact, they often act at multiple and novel target sites (Kostyukovsky et al., 2002; Priestley et al., 2003; Isman, 2006). In terms of their potential to act as sources of commercial insecticides, plant-derived insecticide have received a large amount of attention and this is, in part, because certain essential-oil preparations meet the criteria of reduced-risk insecticides (Isman, 2008);

however, no information is available regarding the potential use of plant essential oils for the control of *M. pruinosa*, even though the insecticidal activity of plant essential oils has been well described by Isman (2000, 2006). Alternatively, information regarding the effects of a variety of essential oils for the control of *M. pruinosa* populations is available (Kim et al., 2013). In this study, the toxicity of the essential oils of cinnamon, its constituents, and structurally related compounds regarding both the nymphs and adults of *M. pruinosa* was evaluated using a direct-contact assay. In addition, the efficacy of experimental spray formulations that contain the oils of the bark and leaf of cinnamon was evaluated against an *M. pruinosa*-adult spray application under field conditions.

#### Materials and Methods

#### Chemicals

The cinnamon essential oils were purchased from UNIQ F&F Co. (Seoul, Korea). 2.4-Dihydrocyl cinnamic acid, αcyanocinnamic acid, a-phenyl cinnamic acid, anethole, benzaldehyde, benzyl cinnamate, caryophyllene, cinnamaldehyde (CAS no. 104-55-2), cinnamyl alcohol, cinnamyl acetate, cinnamyl chloride, estragol, ethyl-hydro cinnamate, (E)-cinnamaldehyde (CAS no. 14371-10-9), hydro-cinnamaldehyde, isoamyl cinnamate, isobutyl cinnamate, methyl cinnamaldehyde, methyl cinnamate, and trans-1-cinnamyl piperazine were purchased from Aldrich (Milwaukee, U.S.A.). Eugenol was purchased from Fluka (Bucks, Switzerland); hydro-cinnamic acid was purchased from Tokyo Chemical Industry (Tokyo, Japan); and limonene was purchased from Wako Pure Chemicals (Osaka, Japan). The surfactant polyoxyethylene + polyoxypropylene (9:1) styrenated phenyl ether (Koremul-SP-1008R®) was provided by Hannong Chemical (Anyang, Korea). All of the other chemicals are of an analytical grade and were commercially available.

#### Insects

Adults and nymphs were collected from seriously damaged host plants at Iksan in Korea from May to August 2015. The collected insects were immediately transported to an insect rearing room (Rural Development Administration, Wanju), where they were transferred to acrylic emergence cages (40  $\times$  40  $\times$  40 cm) that contained the leaf of the rose of Sharon *Hibisscus syriacus*. A species identification of the adult citrus flatid planthopper was then performed in accordance with Kim et al. (2011). The insects were kept in the cages at 25  $\pm$  1°C, and a 60 to 70% relative humidity under a 16 h:8 h light:dark cycle for further bioassays.

#### Experimental spray formulations

Experimental spray formulations comprising cinnamon bark and green leaf oils were prepared for containment in 1,500 mL plastic containers with Homewell polypropylene pump-spray nozzles (Seoul). The emulsifiable formulations contain 0.5% essential oil, 2% of the surfactant polyoxyethylene + polyoxy-propylene (9:1) styrenated phenyl ether, 5% ethanol, and 92.5% water. Single spray applications of the oil preparations were dispensed onto a 100 cm  $\times$  70 cm ginseng field from July to August. All of the treatments were replicated three times.

#### Gas chromatography (GC)

An Agilent 7890A GC System (Palo Alto, U.S.A.) that is equipped with a splitless injector and a flame-ionization-detection system was used to separate and detect the constituents of the cinnamon oils. The constituents were separated with an Agilent J&W Scientific 30 m  $\times$  0.32 mm i.d. ( $d_{\rm f}=0.25~\mu{\rm m})$  HP-5 capillary column (Folsom, U.S.A). The flow rate of the nitrogen carrier gas is 1.0 mL/min. The oven temperature was kept at  $50^{\circ}{\rm C}$  (5 min isothermal), followed by a programming of  $280^{\circ}{\rm C}$  at a rate of  $5^{\circ}{\rm C}$ /min and then an isothermal at  $280^{\circ}{\rm C}$  for 10 min. The injector temperature is  $280^{\circ}{\rm C}$ .

#### Gas chromatography-mass spectroscopy (GC-MS)

A gas chromatography-mass spectrometry (GC-MS) analysis was performed using a Perkin Elmer Clarus 680T gas chromatograph-mass spectrometer (Fort Belvoir, U.S.A.). An Agilent 30 m  $\times$  0.25 mm i.d. ( $d_f$  = 0.5  $\mu$ m) DB-5MS capillary column (Folsom, U.S.A) was also employed. The oven temperature was kept at 50 °C (5 min isothermal), followed by a programming of 280 °C at a rate of 5 °C/min and then an isothermal at 280 °C

for 10 min. The flow velocity of the helium carrier gas is 1.0 mL/min. The ion source temperature is  $250^{\circ}$ C. The interface temperature was kept at  $260^{\circ}$ C, and the mass spectra were obtained at 70 eV. The sector-mass analyzer was set to scan from 35 amu to 550 amu every 0.2 s. The chemical constituents were identified by a comparison of the mass spectra of each peak with those of the authentic samples from a mass spectrum library (NIST mass spectral search program).

#### Bioassay

The toxicity of the test cinnamon oil constituents and the cinnamaldehyde analogues regarding the M. pruinosa nymphs was evaluated by a leaf-dipping assay (Kim et al., 2002). Bioassays were conducted from early April to mid-June, whereby a variety of test-compound concentrations, each in 50  $\mu$ L ethanol, were used; the control received only ethanol-Triton X-100 solution. H. syriacus leaf discs (5.5 cm diameter) were dipped in each test solution for 30 s. After a drying in a fume hood for 2 h, 10 nymphs of M. pruinosa were placed onto the treated- and the control-leaf discs in petri dishes (6 cm diameter  $\times$  1.5 cm height). Each dish was then covered with a lid and sealed with parafilm.

The toxicity of the test cinnamon oil constituents and the cinnamaldehyde analogues regarding the M. pruinosa adults was evaluated by a direct-spray application (Kim et al., 2013). A spray bioassay was used to evaluate the efficacy of 30 compounds regarding the adults. Branches of H. syriacus (4 leaves to 5 leaves), wrapped with water soaked cotton in vials (5 mL), were positioned at the center of rectangular acrylic containers (6.5 × 6.5 × 10 cm), and 10 adults were separately placed onto the leaves. Each of the test compounds were sprayed three times into the rectangular acrylic containers. The containers were then covered with lids.

Treated and control (ethanol-only) nymphs or adults were held under the same conditions as those described above. Test insects were considered to be dead if the body and appendage did not move when prodded with a fine wooden dowel 24 h after treatment. Because all of the bioassays could not be conducted at the same time, the treatments were blocked over time and a separate control treatment was included in each block. Freshly prepared solutions were used for each block of

bioassays (Robertson & Preisler, 1992). All of the treatments were replicated three times using 10 nymphs or adults per replicate.

#### Data analysis

The mortality percentage was transformed into arcsine square-root values for an analysis of variance. The Bonferroni multiple-comparison method was used for the testing of the significant differences among the treatments (SAS Institute, 2004). The means  $\pm$  standard error (SE) of the untransformed data was reported. The concentration mortality data were subjected to a probit analysis (SAS Institute 2004). The LC50 values of the treatments were considered to be significantly different from one another when 95% of the confidence limits failed to overlap.

#### Results

#### Chemical composition of cinnamon oils

The GC and GC-MS analyses enabled the identification of nine compounds that represent the cinnamon oils. The relative concentrations of the identified volatile components are presented in Table 1 according to their elution order on an HP-5 column. The most abundant compounds are (E)-cinnamaldehyde (44.3%) and anethole (5.7%) in the cinnamon oil technical. In the cinnamon green-leaf oil, (E)-cinnamaldehyde (36.4%) and 4,7-bimethyl-benzofuran (10.6%) are the main compounds, whereas in the cinnamon-bark oil, (E)-cinnamaldehyde (35.0%) and estragole (18.7%) are the major compounds.

## Toxicity of cinnamon oil constituents and related compounds regarding nymphs of *Metcalfa pruinosa*

The toxicities of the nine cinnamon oil constituents and the 21 cinnamaldehyde-related compounds regarding the *M. pruinosa* nymphs was evaluated using a leaf-dipping bioassay (Table 2). Based on 24 h LC<sub>50</sub> values, hydro-cinnamic acid (1.55 mg/cm<sup>2</sup>) is the most toxic compound, followed by geranic acid (1.59 mg/cm<sup>2</sup>). The LC<sub>50</sub> values of the cinnamaldehyde, hydro-cinnamaldehyde, (*E*)-cinnamaldehyde, cinnamdyl alcohol, cinnamyl acetate, dibutylphtalate, anethole, a-cyanocinnamic acid, (s)-perillyl alcohol, methyl cinnamaldehyde, and bonyl acetate are between 1.60 mg/cm<sup>2</sup> and 4.94 mg/cm<sup>2</sup>. Low toxicities and no toxicity were observed with the other 15 (5.24 mg/cm<sup>2</sup> to

**Table1.** The chemical composition of three cinnamon oils

C 1	Cinnamon	oil technical	technical Cinnamon		Cinnamon green leaf oil	
Compounds	RT <sup>a</sup>	Area (%)	RT	Area (%)	RT	Area (%)
Malonamic acid	5.343	0.248	-	-	-	-
1-Octadecanamine	7.920	0.149	-	-	-	-
Quinomethionate	8.493	0.147	-	-	-	-
Benzaldehyde	13.745	0.563	13.767	1.160	-	-
Limonene	-	-	-	-	16.645	9.764
2-methyl-Benzofuran	-	-	-	-	23.970	0.361
(E)-Cinnamaldehyde	25.753	44.391	25.759	35.045	25.764	36.350
methyl-Cinnamaldehyde	-	-	-	-	26.448	0.167
4,7-dimethyl-Benzofuran	-	-	-	-	27.650	10.617
Eugenol	-	-	-	-	28.691	7.497
Anethole	30.041	5.749	-	-	-	-
Estragole	-	-	30.057	18.702	30.057	0.303
Caryophyllene	-	-	-	-	30.919	0.169
3-Methoxycinnamaldehyde	-	-	34.104	1.551	-	-
4-Methoxycinnamaldehyde	34.107	0.368	-	-	-	-
2,3-Dimethoxycinnamic acid	-	_	36.902	1.247	_	_

<sup>&</sup>lt;sup>a</sup>Retention time.

Table 2. Toxicity of nine cinnamon oil constituents and (E)-cinnamaldehyderelated compounds regarding nymphs of Metcalfa pruinosa using a leaf-dipping bioassay during a 24 h exposure

Compounds	LC <sub>50</sub> (mg/cm <sup>2</sup> ) (95% CL)	Slope (± SE)	$\chi^{2a}$	<i>P</i> -value
Cinnamaldehyde	1.60 (1.22-2.01)	$2.6 \pm 0.45$	2.93	0.983
Cinnamyl acetate	2.71 (2.11-3.60)	$2.3 \pm 0.41$	4.56	0.918
Anethole <sup>b</sup>	4.09 (3.20-5.16)	$2.6\pm0.43$	6.63	0.954
methyl-Cinnamaldehyde <sup>b</sup>	4.89 (3.98-6.02)	$3.2 \pm 0.47$	4.41	0.926
Eugenol <sup>b</sup>	5.83 (4.52-7.81)	$2.3\pm0.41$	5.06	0.902
Caryophyllene <sup>b</sup>	5.91 (4.78-7.49)	$2.9 \pm 0.46$	3.37	0.971
Estragole <sup>b</sup>	6.35 (5.16-8.07)	$3.1\pm0.48$	4.30	0.932
Limonene <sup>b</sup>	6.38 (5.01-8.56)	$2.4\pm0.43$	2.59	0.989
Benzaldehyde <sup>b</sup>	7.20 (5.62-9.97)	$2.4 \pm 0.43$	3.61	0.963
hydro-Cinnamic acid	1.55 (1.25-1.89)	$3.3\pm0.53$	3.60	0.963
hydro-Cinnamaldehyde	1.97 (1.56-2.45)	$2.8\pm0.45$	4.95	0.893
α-Cyanocinnamic acid	4.15 (3.34-5.14)	$2.9 \pm 0.46$	7.40	0.987
Cinnamyl alcohol	2.26 (1.78-2.86)	$2.6\pm0.43$	3.76	0.957
2,4-Dihydrocyl cinnamic acid	5.74 (4.45-7.76)	$2.3\pm0.41$	5.43	0.960
Ethyl-hydro cinnamate	5.24 (4.19-6.51)	$2.6\pm0.36$	7.49	0.975
Isoamy lcinnamate	5.37 (4.34-6.74)	$2.9\pm0.45$	4.59	0.916
Isobutyl cinnamate	6.06 (4.64-7.86)	$2.1\pm0.31$	5.60	0.959
Methyl cinnamate	6.68 (5.16-9.34)	$2.3\pm0.42$	3.26	0.974
Benzyl cinnamate	9.48 (7.61-11.84)	$2.8 \pm 0.45$	2.90	0.983
Cinnamyl chloride	> 50			
trans-1-Cinnamyl piperazine	> 50			
α-Phenyl cinnamic acid	13.47 (10.65-18.02)	$2.5 \pm 0.44$	3.47	0.968
(E)-Cinnamaldehyde <sup>b</sup>	2.06 (1.51-2.75)	$2.1\pm0.39$	3.62	0.962
(s)-Perillyl alcohol	4.65 (2.79-6.34)	$1.9 \pm 0.41$	2.28	0.993
3-Butylidene	9.41 (7.29-12.30)	$2.3 \pm 0.41$	3.96	0.948
Benzyl benzoate	5.40 (3.75-7.02)	$2.3\pm0.42$	1.36	0.999
Bonyl acetate	4.94 (3.58-6.25)	$2.6 \pm 0.48$	5.75	0.983
Borneol	6.50 (4.69-8.48)	$2.2 \pm 0.41$	6.00	0.981
Dibutylphtalate	3.08 (2.19-4.02)	$2.2 \pm 0.41$	4.23	0.936
Geranic acid	1.59 (1.26-1.95)	$3.1 \pm 0.49$	4.08	0.943

<sup>&</sup>lt;sup>a</sup>Pearson  $\chi^2$ , goodness-of-fit test.

 $13.47 \text{ mg/cm}^2$ ) and two compounds (LC<sub>50</sub> > 50 mg/cm<sup>2</sup>), respectively. The mortalities in the ethanol-treated controls are less than 3%.

#### Toxicity of cinnamon oil constituents and related compounds regarding adults of Metcalfa pruinosa

The toxicities of the 30 compounds regarding the M. pruinosa adults were evaluated using a direct spray bioassay (Table 3). Based on the 24 h LC<sub>50</sub> values, eugenol (10.81 mg) is the most

toxic compound, followed by geranic acid (30.68 mg). The LC<sub>50</sub> values of the cinnamaldehyde, (E)-cinnamaldehyde, hydrocinnamaldehyde, dibutylphtalate, methyl cinnamate, anethole, cinnamyl acetate, hydro-cinnamic acid, methyl cinnamaldehyde, and bonyl acetate are between 59.16 mg and 96.70 mg. Low toxicities and no toxicity were observed with the other 15 (105.44 mg to 255.76 mg) and three compounds (LC<sub>50</sub> > 500 mg), respectively.

<sup>&</sup>lt;sup>b</sup>Originated from cinnamon oils.

**Table 3.** Toxicity of nine cinnamon oil constituents and (*E*)-cinnamaldehyde-related compounds regarding *Metcalfa pruinosa* adults using a direct-contact spray bioassay during a 24 h exposure

Compounds	LC <sub>50</sub> (mg) (95% CL)	Slope (± SE)	$\chi^{2a}$	P-value
Cinnamaldehyde	59.16 (44.94-83.19)	$2.1 \pm 0.40$	1.78	0.997
Cinnamyl acetate	81.37 (63.14-103.84)	$2.4\pm0.42$	1.29	0.999
Anethole <sup>b</sup>	80.42 (57.53-109.68)	$1.8 \pm 0.38$	1.23	0.999
methyl-Cinnamaldehyde <sup>b</sup>	93.84 (69.77-127.97)	$1.9\pm0.38$	1.01	0.999
Eugenol <sup>b</sup>	102.81 (77.61-140.39)	$2.1\pm0.39$	1.91	0.997
Caryophyllene <sup>b</sup>	105.44 (80.94-141.39)	$2.2\pm0.40$	3.51	0.966
Estragol <sup>b</sup>	120.55 (93.09-165.47)	$2.2\pm0.41$	3.10	0.978
Limonene <sup>b</sup>	255.76 (195.95-361.73)	$2.2\pm0.40$	1.57	0.998
Benzaldehyde <sup>b</sup>	151.18 (114.07-228.53)	$2.1 \pm 0.41$	2.03	0.996
(E)-Cinnamaldehyde <sup>b</sup>	64.86 (47.05-84.19)	$2.2\pm0.40$	1.54	0.998
hydro-Cinnamic acid	88.69 (68.19-115.21)	$2.3\pm0.40$	1.89	0.997
hydro-Cinnamaldehyde	67.37 (50.05-86.54)	$2.3\pm0.41$	1.33	0.999
a-Cyanocinnamic acid	148.59 (116.98-204.23)	$2.5\pm0.46$	3.83	0.954
Cinnamdyl alcohol	121.97 (92.19-174.66)	$2.1\pm0.39$	2.06	0.995
2.4-Dihydrocyl cinnamic acid	110.76 (86.04-147.63)	$2.3\pm0.41$	2.69	0.987
Ethyl-hydro cinnamate	193.93 (138.71-281.10)	$1.7\pm0.37$	1.86	0.997
Isoamylcinnamate	131.84 (97.95-199.49)	$1.8\pm0.39$	2.07	0.995
Isobutyl cinnamate	119.01 (89.42-171.08)	$1.9\pm0.39$	3.28	0.973
Methyl cinnamate	79.26 (59.81-102.94)	$2.2\pm0.40$	1.74	0.997
Benzyl cinnamate	233.95 (180.66-319.02)	$2.2\pm0.40$	1.01	0.999
Cinnamyl chloride	> 500			
trans-1-Cinnamyl piperazine	> 500			
a-Phenyl cinnamic acid	> 500			
(s)-Perillyl alcohol	155.51 (122.97-213.61)	$2.6 \pm 0.47$	2.58	0.989
3-Butylidene	227.36 (175.95-306.95)	$2.3\pm0.40$	1.15	0.999
Benzyl benzoate	108.10 (83.49-144.53)	$2.3 \pm 0.41$	3.18	0.976
Bonyl acetate	96.70 (72.57-131.30)	$2.1\pm0.39$	1.32	0.999
Borneol	122.01 (92.20-174.66)	$2.1\pm0.39$	3.66	0.961
Dibutylphtalate	67.49 (48.83-88.57)	$2.1\pm0.40$	3.21	0.975
Geranic acid	30.68 (24.92-37.05)	$3.5 \pm 0.55$	2.63	0.988

<sup>&</sup>lt;sup>a</sup>Pearson  $\chi^2$ , goodness-of-fit test.

#### Efficacy of experimental spray formulations in field

The control efficacy of the experimental spray formulations containing 0.5% cinnamon bark and cinnamon green leaf oils regarding the *M pruinosa* were assayed using the spray-application method in ginseng fields (Table 4). The spray formulations of the cinnamon bark oil and cinnamon green leaf oil resulted in 82.3% and 82.9% mortalities, respectively.

**Table 4.** Toxicity of cinnamon bark and green leaf oil formulations regarding *Metcalfa pruinosa* in ginseng fields

Formulations <sup>a</sup>	Mortality (%) (± SE) <sup>b</sup>		
Cinnamon bark oil	$82.3 \pm 8.7a$		
Cinnamon green leaf oil	$82.9 \pm 5.3a$		

<sup>&</sup>lt;sup>a</sup>0.5% essential oil, 2% polyoxyethylene + polyoxypropylene (9:1) styrenated-phenyl-ether surfactant, 5% ethanol, and 92.5% water

<sup>&</sup>lt;sup>b</sup>Originated from cinnamon oils.

<sup>&</sup>lt;sup>b</sup>Means within a column followed by the same letter are not significantly different at P=0.05 (Bonferroni method).

#### Discussion

Botanical insecticides presently constitute 1% of the world insecticide market (Rozmanet et al., 2007). Many essential oils are known to possess a variety of bio-efficacies such as repellency and deterrence, a reduced palatability, growth inhibition through an altered protein availability, enzyme inhibition, and direct toxicity (Harborne, 1993; Ahn, 2006; Isman, 2006).

Certain plant essential oils and their constituents are capable of manifesting insecticidal activity against different insect species (Isman, 2000; Choi et al., 2003; Kim et al., 2012; Kim et al., 2013) and have been proposed as alternatives. Lee et al. (1997) reported on the toxicity of a range of essential oil constituents in relation to the western corn rootworm (Diabrotica virgifera), the two-spotted spider mite (Tetranychus urticae), and the housefly (Musca domestica), and the dietary effects of a number of monoterpenoids against the European corn borer (Ostrinia nubilalis). Plant essential oils are potential products for the control of M. pruinosa because some of them are selective, biodegrade into nontoxic products, and have lessharmful effects on non-target organisms (Kim et al., 2013; Isman, 2000; 2006).

Our research group (Kim et al., 2013) reported on the toxicity of 124 essential oils regarding M. pruinosa populations. Cinnamon technical, cinnamon green leaf, cinnamon #500, cassia tree, citronella java, and pennyroyal oil all show very strong insecticidal activity regarding M. pruinosa nymph and adult populations. The genus Cinnamomum comprises 250 species that are distributed throughout Asia and Australia (Jayaprakasha et al., 2003). Chang et al. (2001, 2002) found that the leaf essential oil of the cinnamaldehyde type C. osmophloeum has an excellent inhibitory effect against bacteria, termites, mites, mildew, and fungi. Huang and Ho (1998) reported that the methylene chloride extract of cinnamon, Cinnamomum aromaticum (Nees) is insecticidal regarding Tribolium castaneum (Herbst) and Sitophilus zeamais (Motsch); however, there are no prior studies for the M. pruinosa related activity of cinnamomum essential oils and their constituents from Cinnamomum cassia. In the present study, the toxicity of the cinnamon oil constituents and the 21 cinnamaldehyde-related compounds regarding the nymphs and adults of M. pruinosa is confirmed. Hydro-cinnamic acid is the most toxic compound, followed by geranic acid, regarding the nymphs. The toxicity of eugenol is the most toxic compound regarding the adults of M. pruinosa, followed by geranic acid; furthermore, the 5% spray formulations of cinnamon oil provided a sound control efficacy for the ginseng field.

In conclusion, the cinnamon bark and cinnamon green leaf oils could be useful as insecticides for the control of M. pruinosa populations, particularly given their toxicity regarding the corresponding nymphs and adults. To proceed with a practical usage of the essential oils as novel insecticides, further research regarding their safety for humans needs to be established. In addition, changes of the quality of the crops that are treated with these products (e.g., color, flavor, odor, and texture), and the effects on the residues in crops, medicinal plants, and non-target organisms need to be established. Lastly, the completion of detailed tests is necessary to gain an understanding of the way that the insecticidal potency and stability can be improved for an eventual commercial development.

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