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Adulticidal Activity of Phthalides Identified in *Cnidium officinale* Rhizome to B- and Q-Biotypes of *Bemisia tabaci*

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ABSTRACT: The residual contact toxicity of three benzofuranoids (*Z*)-butylidenephthalide (**1**), (3*S*)-butylphthalide (**2**), and (*Z*)-ligustilide (**3**) identified in the rhizome of *Cnidium officinale* (Apiaceae) to B- and Q-biotype females of *Bemisia tabaci* was evaluated using a leaf-dip bioassay. Results were compared with those of eight conventional insecticides. Based on 24 h LC_{50} values, (*Z*)-butylidenephthalide (254 ppm) and (*Z*)-ligustilide (268 ppm) were more toxic than (3*S*)-butylphthalide (339 ppm) against B-biotype females, whereas (*Z*)-ligustilide (254 ppm) and (3*S*)-butylphthalide (338 ppm) were more toxic than (*Z*)-butylidenephthalide (586 ppm) against Q-biotype females. Thiamethoxam, imidacloprid, and acetamiprid differ significantly in toxicity between the B- and Q-biotype females (LC_{50}, 1.7 to 11.6 vs 364.5 to >3000 ppm). This original finding indicates that the phthalides and the neonicotinoids do not share a common mode of action or elicit cross-resistance. Structure−activity relationship indicates that the presence of conjugation rather than aromaticity appeared to play an important role for phthalide toxicities to the B-biotype females. Global efforts to reduce the level of highly toxic synthetic insecticides in the agricultural environment justify further studies on *C. officinale* rhizome-derived materials as potential insecticides for the control of *B. tabaci* populations.

KEYWORDS: *Bemisia tabaci* biotypes B and Q, *Cnidium officinale*, Apiaceae, botanical insecticide, benzofuranoids, phthalides, structure−activity relationship

INTRODUCTION

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is one of the most economically important insect pests because of their cosmopolitan occurrence and abundance in many cropping systems such as cotton, vegetable, and ornamental plants as well as its broad host range (more than 600 plant species).[1] This insect species causes 20−100% yield losses directly from adults and nymphs piercing and sucking sap excessively from the foliage of its host plants and indirectly by deposition of massive quantities of honeydew, which serves as a substrate for fungal infections, and from transmission of numerous plant-pathogenic viruses such as geminiviruses.[2−4] Of 24 described biotypes of *B. tabaci*, the most serious and widespread are the B- and Q-biotypes.[5,6] In South Korea, the B- and Q-biotypes were first identified from collections made in rose and tomato in 1998[7] and cucumber greenhouses in 2005.[8] Recently, the Q-biotype has superseded the B-biotype in most areas of South Korea. Control of *B. tabaci* has been achieved principally by the use of conventional insecticides, including neonicotinoids and insect growth regulators. This insect species has a high tendency to develop resistance to various groups of insecticides[6,9−11] because of its high reproductive potential and short life cycle.[12] Increasing public concern of the environmental effects of insecticides, groundwater contamination, human health effects, crop residues, and undesirable effects on nontarget organisms intensifies when continued or repeated applications of conventional insecticides become necessary. Due to these problems, *B. tabaci* is the target pest of an integrated pest management (IPM) program in which natural enemies and selective insect control agents are key components in greenhouses and in the field. In addition, the use of certain insecticides will likely be reduced in the near future in the United States by the U.S. Environmental Protection Agency as reregistration under the 1996 Food Quality and Protection Act[13] occurs. Reregistration is also occurring under the Commission Regulation (EC) No. 1048/2005 in the European Union[14] and under the Agrochemicals Control Act in South Korea.[15] There is, therefore, a critical need for the development of selective control alternatives with novel target sites in order to establish an efficient management strategy for *B. tabaci*.

Biocides from plants, particularly higher plants, have been suggested as potential alternatives to conventional arthropod control products largely because they constitute a potential source of bioactive chemicals that have been perceived by the general public as relatively safe and pose fewer risks to the environment, with minimal impacts to human and animal health.[16,17] Plant constituents often act at multiple and novel target sites, thereby reducing the potential for resistance.[17−19] Much effort has been focused on them as potential sources of commercial insecticides, in part, because certain plant preparations meet the criteria of reduced risk insecticides.[20] In a preliminary study by the authors, a methanol extract of the rhizome from *Cnidium officinale* Makino (Apiaceae) was shown to have insecticidal activity against the B- and Q-biotype females

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of *B. tabaci*, which were identified by polymerase chain reaction.[21] No information is available concerning the potential use of *C. officinale* extracts for the control of B- and Q-biotypes of *B. tabaci*, although historically it has long been considered to have medicinal properties, such as an analgesic agent in the treatment of cold, headache, rheumatism, and traumatic pains, and against menstrual disorders.[22]

In the current study, we assess the toxicity of the constituents from the rhizome of *C. officinale* against the B- and Q-biotype females of *B. tabaci*. The toxicities of the rhizome constituents were assessed using a leaf-dip bioassay and compared with those of eight currently available insecticides in order to assess their use as future commercial insecticides. The insecticide mode of action and the structure−activity relationship of the constituents are also discussed.

MATERIALS AND METHODS

Instrumental Analyses. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AVANCE 600 spectrometer (Karlsruhe, Germany) using tetramethylsilane (TMS) as an internal standard, and chemical shifts are given in δ (ppm). UV spectra were obtained in methanol with a Kontron UVICON 933/934 spectrophotometer (Milan, Italy), mass spectra on a Jeol GSX 400 spectrometer (Tokyo, Japan), and IR spectra on a Thermo Fisher Nicolet 6700 spectrophotometer (Waltham, MA). Optical rotation was measured with a Rudolph Research Analytical Autopol III polarimeter (Flanders, NJ). An Agilent 1200 series high-performance liquid chromatograph (HPLC) (Santa Clara, CA) was used for the isolation of active principles. Merck silica gel (0.063−0.2 mm) (Darmstadt, Germany) was used for column chromatography. Merck precoated silica gel 60 F_{254} plates were used for analytical thin-layer chromatography (TLC).

Chemicals. The eight insecticides examined in this study were as follows: acetamiprid (99.9% purity), dichlorvos (99.7%), imidacloprid (99.9%), and thiamethoxam (99.7%) purchased from Fluka (Bucks, Switzerland); pirimiphos-methyl (98%), pyridaben (99%), and spinosad (90.5%) purchased from Wako (Osaka, Japan); cypermethrin (96%) purchased from Sigma-Aldrich (St. Louis, MO). Triton X-100 was purchased from Coseal (Seoul, South Korea). All the other chemicals used were of analytical grade and available commercially.

Whiteflies. The stock cultures of the B- and Q-biotypes of *B. tabaci*[21] have been separately maintained in insect rearing rooms to prevent cross-contamination without exposure to any known insecticide. They were reared in acrylic cages (40 × 40 × 40 cm) containing fresh greenhouse-grown cucumber plants, *Cucumis sativus* L. (cv. Baegdadagi). Acrylic cages were held at 25 ± 1 °C and 40−60% relative humidity under a 16:8 h light:dark cycle.

To synchronize the developmental stages for bioassays, adult *B. tabaci* were placed on greenhouse-grown cucumber plants with a Shibata minipump MP-2N (Tokyo, Japan) and allowed to lay eggs for 24 h. After this time, the adults were removed and the infested plants were held at the same conditions as stated above.

Extraction and Isolation. Air-dried rhizome (2 kg) of *C. officinale* was purchased from Boeun medicinal herb shop, Kyoungdong medicinal herb market (Seoul). It was pulverized and extracted with methanol (2 × 3 L) at room temperature for 2 days and filtered. The combined filtrate was concentrated to dryness by rotary evaporation at 40 °C to yield ≈156 g of a dark yellowish oil. The extract was sequentially partitioned into hexane- (24.6 g), chloroform- (11.6 g), ethyl acetate- (7.86 g), butanol- (71.15 g), and water-soluble (40.48 g) portions for subsequent bioassay. The organic solvent-soluble portions were concentrated under vacuum at 40 °C, and the water-soluble portion was concentrated at 50 °C. For isolation of active principles, 1250 and 2500 ppm of each *C. officinale* rhizome-derived material were tested in the leaf-dip bioassay.

The hexane-soluble fraction (10 g) was most biologically active and was chromatographed on a 5.5 × 70 cm silica gel (600 g) column by elution with hexane and ethyl acetate (100:0 (1 L), 100:10 (1.1 L), 80:20 (2 L), 70:30 (1 L). 50:50 (1 L), and 30:70 (1 L) by volume) and finally with methanol (1 L) to provide 32 fractions (each about 250 mL). This procedure was performed two times. Column fractions were monitored by TLC on silica gel plates developed with hexane and ethyl acetate (8:2 by volume) mobile phase. Fractions with similar R_f values on the TLC plates were pooled. Spots were detected by spraying with 10% H_2SO_4 and then heating on a hot plate. The bioactive fractions 13−17 (H1, 6.8 g) and 18−22 (H2, 7.8 g) were obtained. The H1 fraction was rechromatographed on a silica gel column and eluted with hexane and ethyl acetate (100:0 (1 L), 100:10 (2.2 L), 90:10 (2 L), 80:20 (1 L), and 70:30 (1 L) by volume) and finally with methanol (1 L) to give 33 fractions (each about 250 mL). The bioactive fractions 16−22 (H11, 3.97 g) and 23−24 (H12, 0.33 g) were obtained and bioassayed. The H11 and H12 fractions were purified by preparative TLC (hexane: ethyl acetate, 8:2 by volume) to yield compounds **1** (238.2 mg, R_f = 0.64) and **2** (132 mg, R_f = 0.61), respectively. The other active H2 fraction was rechromatographed on a silica gel column using hexane and ethyl acetate (10:1 by volume, 6.1 L) and finally with methanol (1 L). HPLC was used for separation of the constituents from the active fraction (2.3 g). The column was a 4.6 mm i.d. × 150 mm Agilent Eclipse XDB-C18 (Santa Clara, CA) using a mobile phase of acetonitrile and water (7:3 by volume) at a flow rate of 1 mL/min. Chromatographic separations were monitored using a UV detector at 219 nm. Finally, an active principle **3** (263 mg) was isolated at a retention time of 4.21 min.

Leaf-Dip Bioassay. A leaf-dip bioassay[10] was used to evaluate the toxicity of all compounds to the B- and Q-biotype females. Leaves of the greenhouse-grown cucumber plants were collected, and disks (4.5 cm diameter) were punched from each leaf. Test materials, each in 100 μL of ethanol, were suspended in distilled water with Triton X-100 (0.2 g/L). Leaf disks were dipped in each test solution for 30 s. After drying in a fume hood for 50 min, each treated leaf disk was placed on the water-soaked cotton pad in the bottom section of a disposable plastic Petri dish (50 × 15 mm). Groups of 20−30 females (2−3 days old) were separately placed onto the treated leaf disks using the Shibata minipump. The eight insecticides (acetamiprid, imidacloprid, thiamethoxam, cypermethrin, dichlorvos, pirimphos-methyl, pyridaben, and spinosad) served as positive controls and were similarly prepared. Negative controls consisted of the ethanol-Triton X-100 carrier solution in distilled water. The toxicity of each test compound and insecticide was determined with four to five concentrations ranging from 10 to 1000 ppm (for both biotype females) and 0.1 to 1000 ppm (for the B-biotype females) and 1 to 3000 ppm (for the Q-biotype females), respectively.

Treated and control (ethanol-Triton X-100 solution only) females were held at the same conditions as those used for colony maintenance. Whiteflies were considered dead if their bodies and appendages did not move when prodded with fine wooden dowels 24 h posttreatment.[21] Because not all bioassays could be conducted at the same time, treatments were blocked over time with a separate control treatment included in each block.[23] Freshly prepared solutions were used for each block of bioassays. All treatments were replicated 3 times using 20−30 females per replicate.

Data Analysis. Data were corrected for control mortality using Abbott's formula.[24] Mortality percentages were transformed to arcsine square root values for analysis of variance. The Bonferroni multiple-comparison method was used to test for significant differences among the treatments.[25] A *t*-test was used to test for significant differences between two treatments.[25] Means ± standard error (SE) of untransformed data are reported. Concentration−mortality data were subjected to probit analysis.[25] The LC_{50} values of each biotype and their treatments

Table 1. Toxicity of Eight Insecticides to B- and Q-Biotype Females of *Bemisia tabaci* Using Leaf-Dip Bioassay during a 24 h Exposure

	B-biotype				Q-biotype				
insecticide	slope ± SE	LC_{50}, ppm (95% CL)	χ^2	P	slope ± SE	LC_{50}, ppm (95% CL)	χ^2	P	BSR[a]
thiamethoxam	0.6 ± 0.12	1.7 (0.4−3.6)	2.03	0.958		>3000			>1764.7
imidacloprid	0.9 ± 0.13	7.0 (4.3−11.3)	4.34	0.740		>3000			>428.6
acetamiprid	0.8 ± 0.09	11.6 (6.6−19.1)	8.16	0.613	0.8 ± 0.10	364.5 (232.6−579.4)	11.42	0.326	31.4
spinosad	0.6 ± 0.08	24.7 (13.4−44.5)	13.42	0.201	0.6 ± 0.08	33.2 (17.1−65.4)	2.62	0.989	1.3
pyridaben	1.6 ± 0.18	129.2 (91.8−184.5)	3.62	0.822	1.0 ± 0.10	210.9 (139.6−314.3)	1.65	0.998	1.6
dichlorvos	3.6 ± 0.42	182.2 (156.8−208.5)	1.79	0.971	2.4 ± 0.34	223.9 (184.8−271.1)	5.35	0.612	1.2
pirimiphos-methyl	3.2 ± 0.34	188.0 (162.1−214.9)	10.83	0.146	3.3 ± 0.34	235.1 (207.0−266.8)	9.44	0.223	1.3
cypermethrin	2.9 ± 0.28	336.6 (281.0−398.6)	7.31	0.397	2.3 ± 0.28	481.7 (394.8−590.5)	6.93	0.436	1.4

[a] Biotype susceptibility ratio, Q-type LC_{50}/B-type LC_{50}.

Table 2. Toxicity of Fractions from Solvent Partitionings of the Methanol Extract of *Cnidium officinale* Rhizome to B- and Q-Biotype Females of *Bemisia tabaci* Using Leaf-Dip Bioassay during a 24 h Exposure

		mortality, % (mean ± SE)[a]		
material	concn (ppm)	B-biotype	Q-biotype	P-value[b]
methanol extract	2500	100 a	100 a	
	1250	86 ± 1.4 a	51 ± 6.1 cd	0.01
hexane-soluble fr	2500	100 a	100 a	
	1250	99 ± 1.3 a	88 ± 6.9 ab	0.25
chloroform-soluble fr	2500	85 ± 6.7 a	52 ± 8.7 c	0.06
	1250	31 ± 5.7 bc	38 ± 9.2 cde	0.39
ethyl acetate-soluble fr	2500	94 ± 3.5 a	76 ± 8.3 bc	0.12
	1250	48 ± 3.0 b	54 ± 4.4 c	0.18
butanol-soluble fr	2500	30 ± 4.7 bc	35 ± 1.4 cde	0.88
	1250	11 ± 2.2 cd	13 ± 6.9 e	0.93
water-soluble fr	2500	14 ± 3.4 bcd	12 ± 2.1 de	0.75
	1250	7 ± 1.7 d	8 ± 0.2 e	0.13

[a] Means within a column followed by the same letter are not significantly different (P = 0.05, Bonferroni method). [b] According to a t-test.

were considered to be significantly different from one another when their 95% confidence limits (CLs) failed to overlap. The biotype susceptibility ratio (BSR) was determined as the ratio of LC_{50} of Q-biotype females to LC_{50} of B-biotype females.[21]

RESULTS

Insecticide Toxicity. The residual contact toxicity of eight insecticides examined to the B-biotype females of *B. tabaci* was evaluated using the leaf-dip bioassay (Table 1). As judged by 24 h LC_{50} values, thiamethoxam (1.7 ppm) was the most toxic insecticide, followed by imidacloprid, acetamiprid, and spinosad (LC_{50}, 7.0−24.7 ppm). Low toxicity was produced by pyridaben, dichlorvos, and pirimiphos-methyl (LC_{50}, 129.2−188.0 ppm). The toxicity of cypermethrin was the lowest of any of the insecticides. Mortality in the ethanol-Triton X-100-water-treated controls was less than 3%.

The toxic effects of test insecticides on the Q-biotype females were likewise compared (Table 1). Based on 24 h LC_{50} values, spinosad (LC_{50}, 33.2 ppm) was the most effective insecticide. Low toxicity was observed with pyridaben, dichlorvos, pirimiphos-methyl, acetamiprid, and cypermethrin (LC_{50}, 210.9−481.7 ppm). Thiamethoxam and imidacloprid were ineffective.

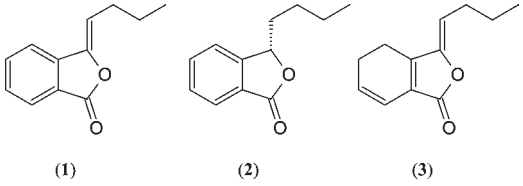

Figure 1. Structures of (*Z*)-butylidenephthalide (**1**), (3*S*)-butylphthalide (**2**), and (*Z*)-ligustilide (**3**).

Bioassay-Guided Fractionation and Isolation. Fractions obtained from the solvent partitionings of the methanol extract of *C. officinale* rhizome were bioassayed against the B- and Q-biotype females, as stated above (Table 2). Significant differences in lethality in fractions of the extract were observed, and they were used to identify peak activity fractions for the next step in the purification. After 24 h of exposure at 1250 ppm, the hexane-soluble fraction was significantly more toxic than the chloroform- and ethyl acetate-soluble fractions against both biotype females. Low lethality was obtained from the butanol- and water-soluble fractions. Significant difference (P = 0.01) in lethality of methanol extract treatment (1250 ppm) was observed against both biotype females.

Leaf-dip bioassay-guided fractionation of *C. officinale* rhizome extract afforded three active principles identified by optical rotation and spectroscopic analyses, including MS and NMR. The three active principles were the benzofuranoids (*Z*)-butylidenephthalide (**1**), (3*S*)-butylphthalide (**2**), and (*Z*)-ligustilide (**3**) (Figure 1). (*Z*)-Butylidenephthalide (**1**): Compound **1** was isolated as an acaricidal principle from *C. officinale* rhizome in our previous work,[26] and the spectral data of compound **1** was largely identical to the published data.[26,27] (3*S*)-Butylphthalide (**2**): yellowish oil; $[\alpha]_D^{15.2}$; −90 (*c* 1.0, $CHCl_3$). UV (EtOH): λ_{max} = 201. EI-MS (70 eV), *m*/*z* (rel int): 190 $[M]^+$ (79), 150 (32), 133 (38), 105 (13), 93 (54), 79 (100), 77 (28). IR (KBr, ν_{max}, cm^{-1}): 1770, 1467, 1176, 733, 699. ^1H NMR ($CDCl_3$, 600 MHz): δ 7.88 (1H, d, J = 7.6 Hz), 7.66 (1H, ddd, J = 7.5, 7.4, 0.9 Hz), 7.51 (1H, dd, J = 7.6, 7.5 Hz), 7.43 (1H, d, J = 7.6 Hz), 5.46 (1H, dd, J = 8.5, 3.8 Hz), 1.72−1.77 (2H, m), 1.24−1.60 (4H, m), 0.91 (3H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 176.6 s, 150.1 s, 133.9 d, 130.3 d, 129.0 s, 126.0 d, 121.0 d, 81.6 d, 34.4 t, 26.8 t, 23.3 t, 13.8 q. (*Z*)-Ligustilide (**3**): yellowish oil;

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Table 3. Toxicity of Three Phthalides to B- and Q-Biotype Females of *Bemisia tabaci* Using Leaf-Dip Bioassay during a 24 h Exposure

	B-biotype				Q-biotype				
compd	slope ± SE	LC_{50}, ppm (95% CL)	χ^2	*P*	slope ± SE	LC_{50}, ppm (95% CL)	χ^2	*P*	BSR[a]
(*Z*)-butylidenephthalide	3.9 ± 0.45	254.2 (223.5−288.9)	7.93	0.339	4.2 ± 0.46	586.3 (527.8−654.7)	2.69	0.912	2.3
(*Z*)-ligustilide	3.5 ± 0.40	268.4 (235.0−309.3)	8.56	0.286	2.0 ± 0.25	254.4 (194.8−321.0)	2.87	0.897	0.9
(3*S*)-butylphthalide	3.1 ± 0.30	338.9 (297.8−385.3)	2.38	0.992	2.6 ± 0.25	338.2 (293.8−389.1)	14.38	0.157	1.0

[a] Biotype susceptibility ratio, Q-type LC_{50}/B-type LC_{50}.

UV (EtOH): λ_{max} = 200. EI-MS (70 eV), *m*/*z* (rel int): 190 $[M]^+$ (55), 161 (47), 148 (50), 134 (10), 105 (50), 77 (45), 55 (100). IR (KBr, ν_{max}, cm^{-1}): 1770, 1668, 1437, 1271, 960. ^1H NMR ($CDCl_3$, 600 MHz): δ 6.29 (1H, dt, *J* = 9.6, 2.0 Hz), 6.01 (1H, dt, *J* = 9.7, 4.3 Hz), 5.22 (1H, t, *J* = 7.9 Hz), 2.60 (2H, t, *J* = 9.7 Hz), 2.50 (2H, q), 2.38 (2H, q), 1.51 (2H, m), 0.96 (3H, t, *J* = 7.4 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.6 s, 148.6 s, 147.0 s, 129.9 d, 124.0 s, 117.1 d, 112.9 d, 28.1 t, 22.4 t, 18.5 t, 13.8 q. The interpretations of proton and carbon signals of compounds **2** and **3** were largely consistent with those of Miyazawa et al.[27] and Tsukamoto et al.,[28] respectively.

Toxicity of Phthalides. The residual contact toxicity of (*Z*)-butylidenephthalide (**1**), (3*S*)-butylphthalide (**2**), and (*Z*)-ligustilide (**3**) to the B-biotype females was evaluated using the leaf-dip bioassay (Table 3). Based on 24 h LC_{50} values, (*Z*)-butylidenephthalide and (*Z*)-ligustilide did not differ significantly in toxicity, but (*Z*)-butylidenephthalide was significantly more toxic than (3*S*)-butylphthalide. The toxicity of (*Z*)-ligustilide and (3*S*)-butylphthalide did not differ significantly from each other. Overall, the toxicity of these phthalides was comparable to that of cypermethrin, but lower than that of either of the three neonicotinoids, spinosad, pyridaben, or the two organophosphorus insecticides (Table 1).

The toxic effects of the three phthalides on the Q-biotype females were likewise compared (Table 3). (*Z*)-Ligustilide and (3*S*)-butylphthalide did not differ significantly in residual contact toxicity but were significantly more toxic than (*Z*)-butylidenephthalide. The toxicities of (*Z*)-ligustilide and (3*S*)-butylphthalide were higher than those of thiamethoxam, imidacloprid, and cypermethrin, but were comparable to those of acetamiprid, pyridaben, dichlorvos, and pirimiphos-methyl. These phthalides were significantly less toxic than spinosad (Table 1).

Biotype Susceptibility. The BSR varied according to chemical examined (Tables 1 and 3). The toxicity was significantly more pronounced in the B-biotype females than in the Q-biotype females to thiamethoxam, imidacloprid, acetamiprid, and (*Z*)-butylidenephthalide because 95% confidence intervals of LC_{50} values of the B-biotype females did not overlap with those of the Q-biotype females. However, there was no significant difference in toxicity to the other five insecticides, (*Z*)-ligustilide, and (3*S*)-butylphthalide between biotypes.

DISCUSSION

Our current findings show that acetamiprid, imidacloprid, and thiamethoxam differ significantly in residual contact toxicity between females of the B- and Q-biotypes of *B. tabaci*. This susceptibility difference might be associated with the development of resistance to the neonicotinoids in the Q-biotype due to the increased use of neonicotinoids against *B. tabaci* in South Korea. The currently used neonicotinoids have failed to control *B. tabaci* in the field and greenhouses, most probably because of the development of resistance.[29] It has been suggested that the Q-biotype may possess a survival advantage over the B-biotype in areas of intensive neonicotinoid use.[30] In addition, the development of resistance to insecticides is more rapid in the Q-biotype populations than in the B-biotype ones,[30] which is in accordance with their higher levels of polymorphism.[31] The results of our present and previous studies may explain why the Q-biotype has almost displaced the B-biotype in most areas in South Korea.

It has been well recognized that certain plant preparations can be developed into products suitable for IPM because some are selective and biodegrade to nontoxic products, as well as can be used in conjunction with biological control.[17] They can be applied to the resting and hiding places of *B. tabaci* in the same manner as conventional insecticides. Some plant preparations manifest insecticidal activity against *B. tabaci*[21,32,33] and have been proposed as alternatives to the conventional insecticides. For example, neem, *Azadirachta indica* A. Juss (Meliaceae), seed-derived materials have a variety of biological activities against various arthropod species without any adverse effects on most nontarget organisms, including natural enemies.[34] *C. officinale* rhizome contains various compounds such as coniferyl ferulate, ferulic acid, phthalides, pregnenolone, sedanonic acid, and tetramethylpyrazine.[22,26−28] In the current study, we used a leaf-dip bioassay to identify the insecticidal constituents of *C. officinale* rhizome against *B. tabaci*. The insecticidal principles were identified as the benzofuranoids (*Z*)-butylidenephthalide (**1**), (3*S*)-butylphthalide (**2**), and (*Z*)-ligustilide (**3**). (*Z*)-Butylidenephthalide and (*Z*)-ligustilide were more toxic than (3*S*)-butylphthalide against the B-biotype females, whereas (*Z*)-butylidenephthalide was least toxic against the Q-biotype females. The residual contact toxicity of these phthalides to the Q-biotype females was significantly higher than that of either imidacloprid or thiamethoxam, and was comparable to that of either acetamiprid, dichlorvos, pirimiphos-methyl, or pyridaben. This original finding indicates that the *C. officinale* rhizome-derived materials may hold promise for the development of novel and effective *B. tabaci* control products even against the insecticide-resistant Q-biotype. However, it has been reported that (*Z*)-butylidenephthalide was 6 times more toxic than (3*S*)-butylphthalide against female *Drosophila melanogaster*, whereas (*Z*)-ligustilide was inactive.[27,28] The difference between our present and previous studies might be attributable to the differences in one or more of physiological or biochemical characteristics between *B. tabaci* and *D. melanogaster*: penetration, detoxifying enzyme activity, and the relative sensitivity to the toxic lesion at the target site.[35,36] In addition, butylidenephthalide possesses acaricidal activity against two house dust mite species, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*.[26]

Structure−activity relationships of chemicals in arthropod pests have been well noted. Tsao et al.[37] reported that enhanced potency of selected monoterpenes and phenols could be achieved through derivatization of the hydroxyl groups, which increased VP (leading to greater fumigant action) and/or increased lipophilicity (leading to better penetration of the insect cuticle and bioavailability in the insect's body). It has been suggested that the numbers and position of the conjugation with the carbonyl group in the lactone ring and aromaticity in the phthalide appeared to play an important role in *Drosophila* larval and adult toxicity.[27,28] In the current study, (*Z*)-butylidenephthalide, which has an aromatic ring and a double bond between the C-3 and C-8 positions in the phthalide, respectively, was significantly more toxic against the B-biotype females than (3*S*)-butylphthalide, which has an aromatic ring and no double bond between the C-3 and C-8 positions in the structure. (*Z*)-Butylidenephthalide was slightly more toxic than (*Z*)-ligustilide, which lacks aromaticity in the structure. These results indicate that the presence of conjugation rather than aromaticity appeared to play an important role for phthalide toxicities to the B-biotype females.

Investigations on the modes of action, the modes of delivery, and the resistance mechanisms of natural insecticides are of practical importance for *B. tabaci* control because they may give useful information on the most appropriate formulations and delivery means to be adapted for their future commercialization and for future resistance management.[16,17] Major mechanisms of resistance to insecticides currently available to control *B. tabaci* are target site insensitivity that reduces sodium channel sensitivity to pyrethroid insecticides[38] or acetylcholinesterase (AChE) sensitivity to organophosphorus insecticides and enhanced metabolism of various groups of insecticides, including neonicotinoids.[6,9,10] Certain plant-derived materials were reported to be highly effective against insecticide- or acaricide-resistant arthropod pests,[39,40] and they are likely to be useful in resistance management strategies. However, available information is limited in *B. tabaci*. In the current study, the residual contact toxicity of (*Z*)-butylidenephthalide, (*Z*)-ligustilide, and (3*S*)-butylphthalide did not differ greatly against both biotype females following the 24 h exposure, whereas acetamiprid, imidacloprid, and thiamethoxam were almost ineffective against the Q-biotype females. These results suggest that the phthalides and the neonicotinoids (agonists of the nicotinic acetylcholine receptor) do not share a common mode of action or elicit cross-resistance. Fumigant activity against adults of *D. farinae* and *D. pteronyssinus* has been reported for butylidenephthalide.[26] In addition, these phthalides were reported to be not *D. melanogaster* AChE inhibitors.[27,28] Detailed tests are needed to fully understand the modes of action of the phthalides, although the octopaminergic and γ-aminobutyric acid receptors have been suggested as novel target sites for some essential oil constituents by Kostyukovsky et al.[18] and Priestley et al.,[19] respectively.

Results of the current study indicate that the *C. officinale* rhizome-derived materials containing the phthalides described herein could be useful in the control of *B. tabaci* populations, particularly in the light of their activity against insecticide-resistant Q-biotypes, because the Q-biotype develops greater insecticide resistance to most of the insecticide groups currently available than the B-biotype.[10] For practical use of the materials as novel insecticides to proceed, or for their use as a component in a push−pull strategy, further research is needed to establish their human safety. In addition, changes in the quality of crops treated with the materials (e.g., color, flavor, odor, and texture) and their effects on nontarget organisms need to be established. Lastly, formulations for improving insecticidal potency and stability, thereby reducing costs, need to be developed.

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