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Repellency and Toxicity of Eight Plant Extracts against the Western Flower Thrips, *Frankliniella occidentalis*

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Abstract: We investigated the repellency and toxicity of eight plant extracts containing celangulin, cnidium lactone, matrine, nicotine, pyrethrins, rotenone, stemonine and veratrine against the western flower thrips (*Frankliniella occidentalis* Pergande; Thysanoptera: Thripidae). Flowers treated with 0.05% nicotine, pyrethrins, stemonine and rotenone harbored fewer western flower thrips in two- and multiple-choice repellency bioassays. When evaluated at concentrations varying from 0.005% to 0.1% in two-choice repellency bioassays, pyrethrins and rotenone were most repellent at 0.01% to 0.1%, and nicotine was most repellent at 0.025%. Mortality was 76% with 0.1% nicotine at 48 h, 78% with 0.5% stemonine at 72 h, and 100% with 0.1% pyrethrins and 0.5% rotenone at 48 h after contact with fresh (<1-d-old) residue. Effective residue age was 1 d for nicotine, pyrethrins and rotenone, and 5 d for stemonine in aged residual toxicity bioassays. Celangulin, cnidium lactone, matrine and veratrine did not provide sufficient repellency and toxicity. Stemonine had inconsistent results. Therefore, additional evaluation of its potential as a botanical insecticide will be needed. When sprayed onto whole plants, plant extracts containing nicotine, pyrethrins, rotenone and stemonine caused unacceptable damage to flowers, suggesting that the final formulation will need to be modified to improve crop safety.

Keywords: botanical insecticides; nicotine; pest management; pyrethrins; rotenone; stemonine

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1. Introduction

Botanical pesticides have long been utilized in arthropod pest management [1,2]. Pyrethrum was used as early as 400 B.C., and commercial products containing nicotine and rotenone were available for pest management since the 17th and 19th centuries, respectively. Azadirachtin was first isolated in 1968 and has since become one of the most widely available and utilized botanical pesticides in the world [3]. Although their importance was largely eclipsed by synthetic pesticides since the mid-20th century, botanical pesticides are enjoying resurgence in popularity because of a renaissance in sustainable and organic agriculture, and ever-increasing concerns over the costs, accessibility, resistance development and environmental impacts of synthetic pesticides [4].

Botanical pesticides currently available in the world's marketplaces are mainly essential oils and raw or refined plant extracts containing secondary metabolites such as flavonoids, terpenoids and phenylpropanoids [2,4,5]. Active ingredients in these botanical insecticides exhibit a range of biological activities, including repellency, neurotoxicity, and inhibition of feeding, growth and reproduction [5,6]. The exact modes of action of these active ingredients are not well understood. Some compounds serve as the progenitors of synthetic pesticides, with the same modes of action [7]. For example, pyrethrins have the same mode of action as pyrethroids, which are sodium channel modulators. Nicotine and neonicotinoids share the same mode of action, which are nicotinic acetylcholine receptor competitive modulators. Other commercialized botanical active ingredients appear to target different processes and proteins within arthropod nervous systems [5].

While numerous studies have investigated the biological activities and efficacies of botanical insecticides, only a limited number have been conducted with the western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), one of the most widely distributed and economically important pests and vectors of tospoviruses in the world. JP503 [an extract of *Perilla frutescens* var. *crispa* (Thunb.) H. Deane; Lamiaceae] caused 100% mortality in a WFT population within 3 h of a leaf dip [8]. Oil and raw extracts from *Azadirachta indica* A. Juss (Meliaceae) and *Datura alba* (Nees) (Solanaceae) caused more than 40% mortality in a WFT population within 48 h [9]. Kiani et al. [10] reported optimal control of a WFT population with a homemade extract of garlic, onion and pepper. Pyrethrins reduced WFT feeding and survival [11]. Oxymatrine or matrine, extracted from the roots of *Sophora flavescens* Aiton (Fabaceae), was slow in killing WFT [12]. UDA-245, an essential oil extract of *Chenopodium ambrosioides* L. (Amaranthaceae), applied at 1% was found to be the only product that had provided suppression of a WFT population at 2 weeks after treatment [13].

New plant-derived active ingredients are frequently being identified and introduced for pharmaceutical and pesticidal uses. The spectrum of insecticidal activity and efficacy of these new active ingredients should be evaluated to assess their potential for incorporation into existing pest management programs to enhance insecticide efficacy or delay insecticide resistance development. We investigated the efficacy of four plant extracts (containing celangulin, cnidium lactone, stemonine and veratrine) against WFT, and compared their efficacies to plant extracts with known activity against thrips (matrine, nicotine, pyrethrins and rotenone). We first evaluated their repellency against WFT in laboratory bioassays. We then evaluated their residual toxicities against WFT in laboratory and greenhouse bioassays. Our goal was to identify novel plant extracts that are more repellent and toxic to WFT than the currently known or available botanical insecticides.

2. Materials and Methods

2.1. Sources of Insects, Plants and Plant Extracts

A colony of WFT was maintained on potted moss-roses (also known as portulacas or purslanes in floricultural trade; *Portulaca grandiflora* Hook., cv. "Happy Hour"; Portulacaceae) in a greenhouse at Clemson University's Pee Dee Research and Education Center (PDREC), Florence, SC, USA. The colony was established with WFT dispersing naturally from the surrounding wheat, cotton and soybean fields into the greenhouse through opened side panels. The colony was maintained by both reproduction of the existing population and individuals dispersing from the fields, and it was never treated with insecticide or fungicide once the colony was established. Infested flowers were collected as needed and allowed to desiccate in Petri dishes (100 × 15 mm) in a laboratory maintained at 21 ± 3 °C, 41 ± 5% R.H., and a 10-h photoperiod. Adult WFT emerging from the desiccating flowers were collected with fine paintbrushes and kept in Petri dishes without any plant materials or moisture for 4 h before the experiments.

All experiments were conducted on Madagascar periwinkle (commonly known as annual vinca in floricultural trade; *Catharanthus roseus* (L.) G. Don, cv. "Cora Red"; Apocynaceae) at PDREC. Periwinkle flowers were chosen for this experiment because of its fully opened and flat petals (which allowed unimpeded observations), as well as its narrow floral tube (which limited refuge for WFT during treatment). Plants were grown from seeds (Parks Wholesale, Greenwood, SC, USA), and germinated in artificial growing media (Sungro Professional; Sun Gro Horticulture, Agawan, MA, USA). Seedlings were grown to about 5 cm in height before they were transplanted into plastic pots (15 cm in diameter, 12 cm in height) filled with growing media. The seedlings were fertilized with 10 g slow-release fertilizer (Osmocote; Scotts Miracle-Gro Company, Marysville, OH, USA) and grown in a greenhouse until they produced flowers and were ready for the experiments. Fully opened flowers excised at the base of pedicels were collected for the laboratory experiments, whereas whole plants were treated and maintained in the greenhouse for the residual toxicity experiment.

Eight plant extracts were purchased from Shaanxi Undersun Biomedtech Co., Ltd. (Xi'an, China). The products were extracted from *Celastrus angulatus* Maxim. (Celastraceae; 6% *w*/*v* celangulin according to the supplier's analysis; same for the other extracts), *Cnidium monnieri* (L.) Cusson (Apiaceae; 10% cnidium lactone), *Derris trifoliata* Lour. (Fabaceae; 5% rotenone), *Nicotiana tabacum* L. (Solanaceae; 90% nicotine), S. *flavescens* (5% matrine), *Stemona japonica* (Blume) Miquel (Stemonaceae; 1% stemonine), *Tanacetum cinerariifolium* (Trevir.) Sch. Bip. (Asteraceae; 70% pyrethrins), and *Veratrum nigrum* L. (Melanthiaceae; 1% veratrine). All plant extracts will be identified henceforth by their respective active ingredients. We did not conduct independent analysis of the plant extracts but relied on non-proprietary information provided by the manufacturer. According to the manufacturer, all plant extracts were prepared in 95% ethanol and processed through the steps of extraction, concentration, sedimentation, filtration, and dilution. The active ingredients were identified and quantified by the manufacturer using HPLC, with laboratory-grade active ingredients as the standards for analysis and comparisons. A proprietary surfactant, 4209-A, was added to all products. The products were kept in a refrigerator until dilution.

Preliminary two-choice bioassays were conducted to confirm that the extraction solution (ethanol) and the adjuvant used in all bioassays in this study (Tween 20) had no repellency against and caused no mortality in WFT. Ethanol (95%) and Tween 20 (97% polyoxyethylene 20-sorbitan monolaurate) were diluted to 0.05% *v*/*v* of final solution. Periwinkle flowers were treated with deionized water, ethanol or Tween 20 solution and exposed to adult WFT in two-choice bioassays (water vs. ethanol and water vs. Tween 20) as described in the next section. The data were analyzed as described in the next section. In the preliminary bioassays, the numbers of live WFT at 24 h after insect introduction were not significantly different among the water-, ethanol- and Tween 20-treated flowers ($p > 0.05$), suggesting that the extraction solution and adjuvant did not influence the repellency and mortality observed in the subsequent bioassays.

2.2. Two-Choice Repellency Bioassays: All Plant Extracts

Commercial products of most active ingredients evaluated in this study are not available in the USA and, therefore, could not be used to inform the test concentration. Therefore, we chose to initiate this study using the test concentration of 0.05% for all plant extracts based on the most commonly labeled application concentration of commercial insecticides with pyrethrins (e.g., EverGreen Pyrethrum Concentrate with 5% pyrethrins; McLaughlin Gormlet King Company, Minneapolis, MN, USA). All plant extracts were diluted to 0.05% active ingredient with water. Tween 20, also diluted to 0.05% of final solution, was added as an adjuvant to all solutions. Tween 20-only solution was used as the control.

Periwinkle flowers were dipped individually and completely in a solution of plant extract or Tween 20 for 5 to 8 sec and air-dried for 30 min. The treated flowers were inserted individually through a hole drilled at the bottom of a plastic container (15 cm in length, 15 cm in width, and 12 cm in height), with the pedicels immersed completely in a bowl of water to maintain vigor. Each plastic container enclosed one treated flower and one control flower arranged opposite of each other. Ten adult WFT were introduced in the center of each container and allowed to disperse. The containers were covered with plastic sheets (with small ventilation holes punctured throughout) that were attached with rubber bands to prevent escape of WFT. Each extract-control pair was replicated eight (celangulin, cnidium lactone, matrine, pyrethrins and rotenone treatments) or 12 times (nicotine, stemonine and veratrine treatments).

The numbers of live WFT on each treated and control flower were recorded at 0.5, 3, 6, 24 and 48 h after release. The hypothesis of equal proportions of WFT on the treated and control flowers (i.e., 50–50%) at each sampling time was tested with binomial exact tests at $\alpha = 0.05$ [14].

2.3. Multiple-Choice Repellency Bioassays: Selected Plant Extracts

Four plant extracts that demonstrated the strongest repellency in the two-choice repellency bioassays in the previous section were selected for this experiment. These plant extracts and Tween 20 were diluted to 0.05% active ingredient, and the flowers were prepared and treated as previously described. In a plastic container (described above), the treated and control flowers were arranged in random order in a circle (equal distance between two adjacent flowers, and from each flower to the center of the container). Ten adult WFT were released and observed as previously described. Each multiple-choice combination was replicated five times.

The hypothesis of equal proportions of WFT on the treated and the control flowers (i.e., 20% on each flower) was tested with Chi-Square Goodness-of-Fit tests at $\alpha = 0.05$ [14]. When significant difference among plant extracts and the control was detected, the means were separated with distribution-free Bonferroni Multiple Comparison [14].

2.4. Two-Choice Repellency Bioassays: Concentrations

Four plant extracts that demonstrated the strongest repellency in the two-choice repellency bioassays were selected for this experiment aimed at identifying the most effective concentration. These plant extracts were diluted to 0.005, 0.01, 0.025, 0.05 and 0.1% active ingredient. Tween 20 was diluted to 0.05% and served as the control. The flowers were treated and prepared as previously described, and one treated flower of a specific concentration and one control flower were arranged opposite of each other in a plastic container. Ten adult WFT were released and observed as previously described. All treated-control pairs were replicated six times, except for the nicotine-control pair, which was replicated seven times.

The hypothesis of equal proportions of WFT on the treated and control flowers at each sampling time was tested with binomial exact tests at $\alpha = 0.05$ [14]. Proportion of WFT repelled by each plant extract at a specific concentration was calculated (i.e., difference in WFT abundance between the treated and control flowers divided by the total WFT abundance on the treated and control flowers). The data were arcsine-transformed, then analyzed with two-factor repeated measure analysis of variance (ANOVA) (with plant extract and concentration as the main factors, and observation time as the repeated factor) [14]. When Sphericity Tests indicated that $p > \chi^2$ was <0.05, the Hutnh-Feldt-Lecoute adjusted p values were used. Percent repellency was calculated as [(number of thrips on control flower–number of thrips on treated flower)/total number of thrips on flowers] $\times$ 100%. The maximum percent repellency (when data from all observation times were pooled was arcsine-transformed and subjected to two-way ANOVA (at $\alpha = 0.05$) to detect differences among plant extracts and concentrations [14].

2.5. Toxicity Bioassays: Fresh Residue

All eight plant extracts were diluted to 0.01, 0.05, 0.1 and 0.5% active ingredient with water and Tween 20 (0.05%). Tween 20 solutions diluted to the above concentrations were used as the untreated control. This experiment was conducted as a no-choice test. A group of five fully opened flowers, Petri dishes (9 cm in diameter) and chiffon mesh (16 $\times$ 16 cm) were dipped in plant extract solution for 5 to 8 sec and air-dried for 30 min. Each flower was transferred to a treated Petri dish, with the pedicel inserted into a cup of water through a hole drilled at the bottom of the Petri dish. Ten adult WFT were introduced into each Petri dish and covered with a treated chiffon mesh (held in place with a rubber band) to prevent escape.

The numbers of live WFT on each flower were recorded at 24, 48 and 72 h after treatment. Since mortality >5% was observed in the control, mortality of WFT in each plant extract treatment at a specific concentration was corrected with Abbott's formula [15]. Replicates of any plant extract-concentration combination that returned negative values in Abbott-corrected mortality were not included in the data analysis. Abbott-corrected mortality data were arcsine-transformed and analyzed with two-factor repeated measure ANOVA (with plant extract and concentration as the main factors, and observation time as the repeated factor) at $\alpha = 0.05$ [14].

When the assumption of Sphericity Test was violated, Huynh-Feldt-Lecoutre adjusted p values were used to detect significant differences. Within each plant extract and observation time, Fisher's least significant difference (LSD) test ($\alpha = 0.05$) was used to separate mean percent mortality among concentration treatments [14].

2.6. Toxicity Bioassay: Aged Residue

Specific concentrations of nicotine (0.1%), pyrethrins (0.05%), rotenone (0.1%) and stemonine (0.5%) were selected for this experiment based on their abilities to achieve >75% (Abbott-corrected) mortality in the fresh residue toxicity bioassays. Tween 20 solution (0.05%) was used to dilute all plant extracts and served as the untreated control. Each solution was sprayed onto a group of six potted flowering periwinkle plants maintained in a greenhouse with a compressed-CO_2 sprayer (at 240 kPa) fitted with a flat-fan nozzle (TP8004VS; TeeJet Technologies, Springfield, IL, USA) and at an application volume of 935 L/ha. Petri dishes (with a hole drilled in the bottom) and chiffon mesh (16 × 16 cm) were sprayed at the same time and aged so that, when WFT were introduced into the arenas, all surfaces contacted by the thrips contained residue of the same age. Senescing flowers and unopened flower buds were removed from the plants before treatment. Flower buds were continuously removed throughout the experimental period, so that only treated opened flowers remained. The plants, Petri dishes and chiffon mesh were kept in the greenhouse for 1, 3, 5 and 7 d before fully opened flowers were collected and used in the experiment. The greenhouse was maintained on average 33.9 °C (23.0 °C to 38.9 °C), 50% R.H. (36% to 84%), and 35% shade (achieved with shade clothes). Phytotoxicity (percentage of flower or leaf surface area showing discoloration or bleaching) on leaves and flowers of plants treated by all treatments were recorded daily for 3 d after the application.

The aged residue toxicity bioassay was conducted as a no-choice test. Fully opened, treated flowers were collected and brought back to the laboratory, where they were transferred individually into treated Petri dishes as previously described. Ten adult WFT were introduced onto a flower with residue of one plant extract of a specific residual age and covered with treated chiffon mesh. Live WFT on each flower was counted at 24, 48 and 72 h after introduction.

Proportion of WFT killed in each Petri dish was calculated and corrected with Abbott's formula [15], after which the data were arcsine-transformed and analyzed with two-factor repeated measure ANOVA (with plant extract and residual age as the main factors and observation time as the repeated factor) at $\alpha = 0.05$ [14]. When the assumption of Sphericity Test was violated, Huynh-Feldt-Lecoutre adjusted p values were used to detect significant differences. Fisher's LSD test ($\alpha = 0.05$) was used to separate mean percent mortality among concentration treatments within each plant extract and residual age combination [14].

3. Results

3.1. Repellency

Among periwinkle flowers treated with 0.05% solutions of the eight plant extracts in the two-choice repellency (all plant extracts) bioassays, only those treated with nicotine, rotenone, pyrethrins and stemonine harbored significantly fewer WFT at one or more observation times than flowers treated with 0.05% Tween 20 solution (Table 1). Significantly fewer WFT were found on flowers treated with rotenone and pyrethrins at all times. Although the numbers of WFT on stemonine- and nicotine-treated flowers were lower than those on the Tween 20-treated flowers at all times, significant differences were detected only at 3 and 6 h after treatment for stemonine, and 3 and 48 h after treatment for nicotine. The numbers of WFT on cnidium lactone-, celangulin-, matrine- and veratrine-treated flowers were not significantly different from those on the Tween 20-treated flowers at any time after introduction in the two-choice repellency (all plant extract) bioassays.

Table 1. Mean numbers (±SEM) adult western flower thrips observed on periwinkle flowers dipped in solution of Tween-20 (control) and one plant extract in two-choice repellency (plant extracts) bio-assays.

Active Ingredient	Time after Introduction (hour)	Number on Control Flower	Number on Treated Flower	Z Value [1]	Two-Sided *p* Value
Celangulin	0.5	1.4 ± 0.3	2.1 ± 0.9	−1.1339	0.2568
	3	1.5 ± 0.5	2.3 ± 0.5	−1.0954	0.2733
	6	1.4 ± 0.4	1.8 ± 0.4	−0.6000	0.5485
	24	4.0 ± 0.8	3.0 ± 0.8	1.0690	0.2850
	48	2.7 ± 0.8	2.8 ± 0.9	−0.1741	0.8618
Cnidium lactone	0.5	1.8 ± 0.6	1.3 ± 0.6	0.8165	0.4142
	3	2.0 ± 0.5	2.6 ± 0.5	−0.8220	0.4111
	6	2.0 ± 0.5	1.0 ± 0.4	1.6330	0.1025
	24	2.4 ± 0.6	1.6 ± 0.4	1.0607	0.2888
	48	3.3 ± 0.9	2.0 ± 0.7	1.4142	0.1573
Matrine	0.5	1.8 ± 0.4	1.1 ± 0.5	1.0426	0.2971
	3	2.0 ± 0.5	2.6 ± 0.5	−0.8220	0.4111
	6	2.6 ± 0.7	3.5 ± 0.5	−1.0000	0.3173
	24	2.9 ± 0.7	4.4 ± 0.8	−1.5757	0.1151
	48	3.0 ± 0.7	3.3 ± 0.8	−0.3244	0.7456
Nicotine	0.5	1.6 ± 0.4	0.9 ± 0.6	1.4606	0.1441
	3	1.8 ± 0.7	0.8 ± 0.3	2.3349	0.0196
	6	2.6 ± 0.6	1.6 ± 0.5	1.6971	0.0897
	24	3.5 ± 0.6	2.5 ± 0.6	1.4142	0.1573
	48	3.4 ± 0.8	1.7 ± 0.3	2.3805	0.0173
Pyrethrins	0.5	1.8 ± 0.8	0	3.7417	<0.0001
	3	1.8 ± 0.5	0.4 ± 0.3	2.6679	0.0076
	6	2.1 ± 0.5	0.5 ± 0.2	2.8368	0.0046
	24	3.9 ± 0.9	0.4 ± 0.2	4.8020	<0.0001
	48	4.3 ± 1.3	1.7 ± 0.4	2.6667	0.0077
Rotenone	0.5	1.8 ± 0.5	0.4 ± 0.3	2.6679	0.0076
	3	2.6 ± 0.8	0.9 ± 0.5	2.6458	0.0082
	6	2.5 ± 0.6	0.5 ± 0.3	3.2660	0.0011
	24	3.3 ± 0.7	0.5 ± 0.2	4.0166	<0.0001
	48	2.8 ± 0.6	0.8 ± 0.5	2.5584	0.0150
Stemonine	0.5	2.1 ± 0.6	1.2 ± 0.3	1.7614	0.0782
	3	3.3 ± 0.9	1.3 ± 0.4	3.2660	0.0011
	6	3.8 ± 0.7	1.6 ± 0.4	3.2500	0.0012
	24	2.7 ± 0.5	2.0 ± 0.5	1.0690	0.2850
	48	1.9 ± 0.6	1.6 ± 0.4	0.5071	0.6121
Veratridine	0.5	1.6 ± 0.4	1.8 ± 0.3	−0.4685	0.6394
	3	2.3 ± 0.6	2.3 ± 0.7	0	1.0000
	6	3.3 ± 0.8	2.8 ± 0.6	0.8193	0.4126
	24	3.4 ± 0.7	3.8 ± 0.7	−0.1048	0.9165
	48	4.0 ± 0.7	3.3 ± 0.5	0.8193	0.4126

[1] The hypothesis of equal numbers of thrips between the control and treated flowers was tested with binomial exact tests at $\alpha = 0.05$. Degree-of-freedom was not generated for binomial exact tests.

Based on their performance in the two-choice repellency (all plant extracts) tests, nicotine, pyrethrins, rotenone and stemonine were selected for the multiple-choice repellency (plant extracts) bioassays. In the multiple-choice bioassays, flowers treated with the four plant extracts consistently harbored numerically fewer WFT than Tween 20-treated flowers at all observation times, but the repellencies among plant extract treatments were not always significantly greater than the control (Table 2). All selected plant extracts appeared to repel WFT as early as 3 h after treatment, but only the pyrethrins treatment resulted in consistently and significantly lower WFT abundance than the control at all times. The repellency of rotenone and stemonine were slightly lower than that of pyrethrins and

greater than that of nicotine at most times. The differences in the numbers of WFT between treated and control flowers were significant only at 3 and 6 h for stemonine, 6 and 48 h for nicotine, and 6 to 48 h for rotenone.

Table 2. Mean numbers (±SEM) of adult western flower thrips observed on periwinkle flowers treated with selected plant extracts and Tween 20 (control) at varying times after introduction in multiple-choice repellency bioassays.

Active Ingredient	Time after Introduction (hour) [1]				
	0.5	**3**	**6**	**24**	**48**
Nicotine	0.5 ± 0.2 ab	0.9 ± 0.4 ab	1.5 ± 0.4 b	1.8 ± 0.7 ab	1.9 ± 0.6 b
Pyrethrins	0.1 ± 0.1 b	0.4 ± 0.2 b	0.3 ± 0.1 c	0.1 ± 0.1 c	0.5 ± 0.2 c
Rotenone	0.5 ± 0.3 ab	0.6 ± 0.2 ab	0.6 ± 0.2 bc	0.5 ± 0.2 bc	0.4 ± 0.2 c
Stemonine	0.3 ± 0.1 ab	0.5 ± 0.2 b	0.3 ± 0.2 c	1.6 ± 0.4 ab	2.5 ± 0.5 ab
Tween 20	1.1 ± 0.5 a	1.8 ± 0.5 a	2.6 ± 0.6 a	2.1 ± 0.6 a	3.7 ± 0.6 a
χ^2	16.3784	24.5938	55.6203	39.7143	64.5075
d.f.	4	4	4	4	4
P	0.0026	<0.0001	<0.0001	<0.0001	<0.0001

[1] The hypothesis of equal numbers of thrips among the treated and control flowers was tested with chi-square goodness-of-fit tests at $\alpha = 0.05$. Means in a column (specific hour after introduction) followed by the same letters are not significantly different among active ingredients by distribution-free Bonferroni Multiple Comparison $\alpha = 0.05$.

The repellency of nicotine, rotenone, pyrethrins and stemonine diluted to 0.005, 0.01, 0.025, 0.05 and 0.1% were further evaluated in two-choice repellency (concentrations) bioassays. Flowers treated with pyrethrins and rotenone solutions diluted to 0.01% to 0.1% consistently harbored significantly fewer WFT than Tween 20-treated (control) flowers at all observation times, but the differences in the numbers of WFT on treated and control flowers at 0.005% active ingredient were not significant for rotenone and inconsistent for pyrethrins (Table 3). Stemonine consistently failed to repel WFT and resulted in similar abundances of WFT on treated and control flowers at almost all concentrations and observation times. Nicotine diluted to 0.005 and 0.01% was not effective in repelling WFT, whereas rates of 0.05 and 0.1% were less repellent than 0.025%.

Table 3. Mean numbers (±SEM) adult western flower thrips observed on periwinkle flowers treated with Tween 20 (control) and with a plant extract at specific concentration in two-choice repellency (concentrations) bioassays.

Active Ingredient	Concentration (%)	Time Since Introduction (hour)	Number on Control Flower	Number on Treated Flower	Z Value [1]	Two-Sided *p* Value
Pyrethrins	0.005	0.5	1.5 ± 0.6	0.5 ± 0.3	−1.7321	0.0833
		3	2.8 ± 1.0	1.3 ± 0.6	−1.8000	0.0719
		6	3.8 ± 0.6	1.5 ± 0.8	−2.4749	0.0133
		24	3.2 ± 0.7	2.2 ± 0.7	−1.0607	0.2888
		48	3.0 ± 0.4	1.3 ± 0.4	−1.9612	0.0499
	0.01	0.5	1.3 ± 0.4	0.2 ± 0.2	−2.3333	0.0196
		3	2.8 ± 0.5	1.0 ± 0.4	−2.2937	0.0218
		6	3.8 ± 0.7	1.2 ± 0.3	−2.9212	0.0035
		24	3.3 ± 0.8	1.3 ± 0.4	−2.2678	0.0233
		48	4.0 ± 0.7	1.7 ± 0.3	−2.4010	0.0164
	0.025	0.5	0.8 ± 0.3	0	2.2361	0.0253
		3	2.2 ± 0.5	0.2 ± 0.2	−3.2071	0.0013
		6	3.3 ± 0.3	0.7 ± 0.3	−3.2660	0.0011
		24	2.8 ± 0.5	0.5 ± 0.2	−3.1305	0.0017
		48	2.8 ± 0.6	0.2 ± 0.2	−3.7712	0.0002
	0.05	0.5	1.5 ± 0.3	0.2 ± 0.2	−2.5298	0.0114
		3	2.3 ± 0.7	0.3 ± 0.2	−3.0000	0.0027
		6	3.5 ± 0.8	0.2 ± 0.2	−4.2640	<0.0001
		24	2.7 ± 0.5	0.7 ± 0.3	−2.6833	0.0073
		48	2.5 ± 0.4	0.7 ± 0.7	−2.5236	0.0116
	0.1	0.5	0.8 ± 0.5	0	2.2361	0.0253
		3	2.2 ± 0.7	0.2 ± 0.2	−3.2071	0.0013
		6	2.7 ± 0.8	0	4.0000	<0.0001
		24	3.2 ± 0.3	0.2 ± 0.2	−4.0249	<0.0001
		48	3.5 ± 0.7	0.3 ± 0.2	−3.9618	<0.0001

Table 3. *Cont.*

Active Ingredient	Concentration (%)	Time Since Introduction (hour)	Number on Control Flower	Number on Treated Flower	Z Value [1]	Two-Sided *p* Value
Rotenone	0.005	0.5	1.2 ± 0.5	0.3 ± 0.2	−1.6667	0.0956
		3	1.2 ± 0.3	0.7 ± 0.2	−0.9045	0.3657
		6	1.8 ± 0.6	1.3 ± 0.6	−0.6882	0.4913
		24	2.7 ± 0.8	2.0 ± 0.6	−0.7559	0.4497
		48	2.2 ± 0.4	1.8 ± 0.2	−0.4082	0.6831
	0.01	0.5	1.8 ± 0.4	0	3.3166	0.0009
		3	3.7 ± 0.4	1.2 ± 0.3	−2.7854	0.0053
		6	4.5 ± 0.8	1.5 ± 0.4	−3.0000	0.0027
		24	3.2 ± 0.5	1.2 ± 0.5	−2.3534	0.0186
		48	3.7 ± 0.3	1.7 ± 0.3	−2.1213	0.0339
	0.025	0.5	1.3 ± 0.8	0.3 ± 0.2	−1.8974	0.0578
		3	3.0 ± 1.0	0.8 ± 0.3	−2.7107	0.0067
		6	3.7 ± 0.9	1.2 ± 0.4	−2.7854	0.0053
		24	3.8 ± 0.6	0.5 ± 0.5	−3.9223	<0.0001
		48	4.7 ± 0.6	0.7 ± 0.3	−4.2426	<0.0001
	0.05	0.5	1.5 ± 0.5	0.7 ± 0.3	−1.3868	0.1655
		3	3.3 ± 0.8	1.3 ± 0.6	−2.2678	0.0233
		6	3.2 ± 0.9	1.2 ± 0.5	−2.3534	0.0186
		24	3.5 ± 0.7	1.0 ± 0.4	−2.8868	0.0039
		48	3.3 ± 0.6	0.7 ± 0.3	−3.2660	0.0011
	0.1	0.5	1.3 ± 0.8	0.2 ± 0.2	−2.3333	0.0196
		3	2.3 ± 0.6	0.8 ± 0.5	−2.0647	0.0389
		6	2.2 ± 0.7	0.5 ± 0.2	−2.5000	0.0124
		24	3.2 ± 0.7	0.2 ± 0.2	−4.0249	<0.0001
		48	3.5 ± 0.8	0.3 ± 0.3	−3.9618	<0.0001
Nicotine	0.005	0.5	1.3 ± 0.5	0.9 ± 0.5	−0.7746	0.4386
		3	1.0 ± 0.4	2.3 ± 0.8	1.8766	0.0606
		6	1.4 ± 0.5	2.3 ± 0.6	1.1767	0.2393
		24	3.0 ± 0.6	2.3 ± 0.4	−0.8220	0.4111
		48	2.3 ± 0.4	1.3 ± 0.3	−1.4000	0.1615
	0.01	0.5	1.0 ± 0.4	1.3 ± 0.7	0.5000	0.6171
		3	2.0 ± 0.8	2.0 ± 0.5	0	1.0000
		6	2.4 ± 0.8	3.3 ± 0.9	0.9487	0.3428
		24	2.6 ± 0.5	3.6 ± 0.9	1.0675	0.2858
		48	2.3 ± 0.5	3.4 ± 0.4	1.2649	0.2059
	0.025	0.5	2.0 ± 0.9	0.1 ± 0.1	−3.3566	0.0008
		3	2.1 ± 0.8	0.9 ± 0.4	−1.9640	0.0495
		6	3.3 ± 1.0	1.3 ± 0.5	−2.4749	0.0133
		24	2.7 ± 0.7	1.1 ± 0.3	−2.1170	0.0343
		48	3.1 ± 0.5	1.3 ± 0.4	−2.3349	0.0196
	0.05	0.5	1.1 ± 0.6	0.7 ± 0.4	−0.8321	0.4054
		3	3.0 ± 0.8	1.6 ± 0.6	−1.7678	0.0771
		6	2.3 ± 0.8	1.9 ± 0.5	−0.5571	0.5775
		24	3.4 ± 0.6	2.0 ± 0.5	−1.6222	0.1048
		48	2.9 ± 0.5	1.9 ± 0.3	−1.2185	0.2230
	0.1	0.5	1.0 ± 0.4	0.4 ± 0.4	−1.2649	0.2059
		3	2.7 ± 0.7	1.0 ± 0.3	−2.3534	0.0186
		6	3.3 ± 0.7	1.1 ± 0.4	−2.6941	0.0071
		24	2.6 ± 0.4	2.1 ± 0.6	−0.5222	0.6015
		48	3.4 ± 0.5	2.0 ± 0.6	−1.6222	0.1048
Stemonine	0.005	0.5	0.5 ± 0.3	1.3 ± 0.8	1.5076	0.1317
		3	2.0 ± 0.9	2.3 ± 0.6	0.3922	0.6949
		6	3.0 ± 1.1	2.3 ± 0.5	−0.7071	0.4795
		24	2.7 ± 1.0	2.0 ± 0.4	−0.7559	0.4497
		48	2.7 ± 0.7	1.7 ± 0.5	−1.1767	0.2393
	0.01	0.5	0.8 ± 0.3	1.0 ± 0.4	0.3015	0.7630
		3	3.2 ± 1.5	1.2 ± 0.4	−2.3534	0.0186
		6	2.8 ± 0.5	0.5 ± 0.2	−3.1305	0.0017
		24	3.0 ± 0.6	2.2 ± 0.5	−0.8980	0.3692
		48	2.3 ± 0.6	2.2 ± 0.5	−0.1925	0.8474
	0.025	0.5	1.0 ± 0.6	0.7 ± 0.4	−0.6325	0.5271
		3	1.5 ± 0.6	1.3 ± 0.5	−0.2425	0.8084
		6	2.2 ± 0.5	1.2 ± 0.3	−1.3416	0.1797
		24	2.0 ± 0.6	1.2 ± 0.5	−1.1471	0.2513
		48	2.2 ± 0.5	1.7 ± 0.4	−0.6255	0.5316
	0.05	0.5	1.3 ± 0.7	1.7 ± 1.1	0.4714	0.6374
		3	1.3 ± 0.6	2.0 ± 0.5	0.8944	0.3711
		6	1.3 ± 0.6	2.3 ± 0.6	1.2792	0.2008
		24	1.8 ± 0.5	2.2 ± 0.7	0.4082	0.6831
		48	1.8 ± 0.5	2.2 ± 0.7	0.4082	0.6831
	0.1	0.5	1.3 ± 0.7	1.0 ± 0.6	−0.5345	0.5930
		3	2.3 ± 0.3	1.2 ± 0.4	−1.5275	0.1266
		6	2.5 ± 0.8	1.3 ± 0.4	−1.4596	0.1444
		24	2.8 ± 0.7	2.0 ± 1.1	−0.9285	0.3532
		48	3.0 ± 1.3	1.5 ± 0.7	−1.7321	0.0833

[1] The hypothesis of equal numbers of thrips between the control and treated flowers was tested with binomial exact tests at $\alpha = 0.05$. Degree-of-freedom was not generated by binomial exact tests.

Two-factor repeated measure analysis suggested that both plant extracts (F = 9.69, d.f. = 3, 64, $p < 0.0001$) and concentrations (F = 2.83, d.f. = 4, 64, $p = 0.0318$) significantly influenced the repellency against WFT in the two-choice repellency (concentrations) tests, but observation times did not (F = 1.77, d.f. = 4, 256, $p = 0.1535$). Except for the interaction between observation times and plant extracts (F = 2.19, d.f. = 12, 256, $p = 0.0246$), all other two- or three-way interactions among plant extracts, concentrations and observation times were not significant ($p > 0.05$). The maximum percent repellency (across all observation times) of pyrethrins and rotenone were greater than those of nicotine and stemonine (Extract: F = 13.14, d.f. = 3, 99, $p < 0.0001$; Concentration: F = 2.22, d.f. = 4, 99, $p = 0.0726$; Extract × Concentration: F = 0.58, d.f. = 12, 99, $p = 0.8571$) (Figure 1). Both pyrethrins and rotenone achieved 100% repellency at 0.01%, whereas the greatest repellency was observed at 0.025% and 0.1% for nicotine and stemonine, respectively.

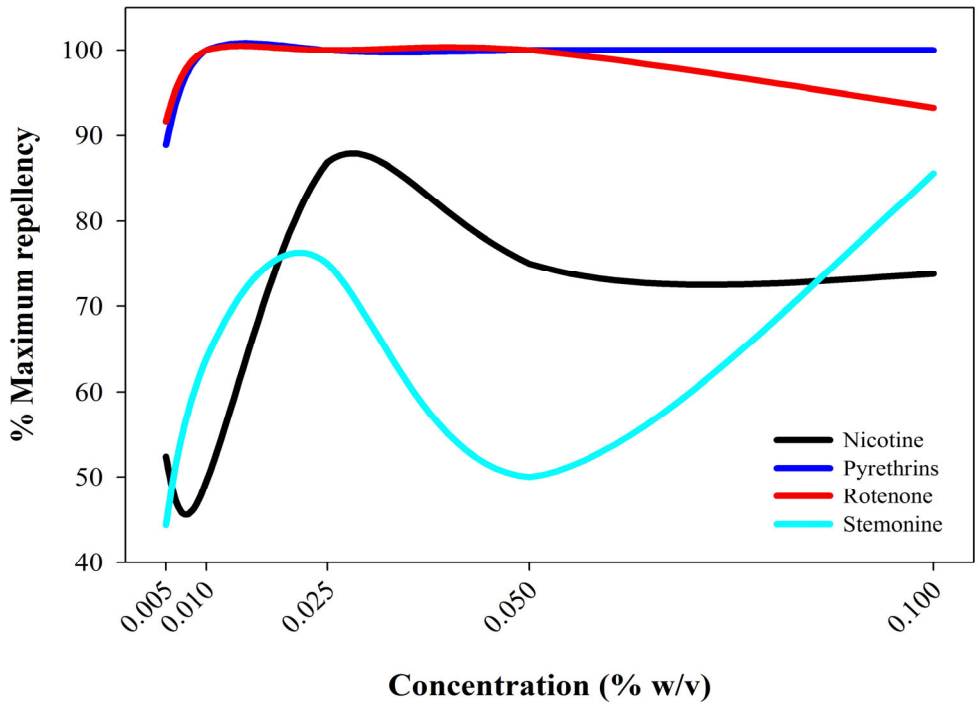


Figure 1. Mean maximum percent repellency achieved by varying concentrations of plants extracts in the two-choice repellency, multiple-concentrations bioassays.

3.2. Fresh Residue Toxicity

Percent mortality of adult WFT on Tween 20-treated flowers averaged 0% to 14% at the four tested concentrations. Abbott-corrected percent mortality in the fresh residue toxicity bioassay differed significantly among active ingredients (F = 146.33, d.f. = 7, 112, $p < 0.0001$), concentrations (F = 103.13, d.f. = 3, 112, $p < 0.0001$), observation times (F = 84.51, d.f. = 2, 224, $p < 0.0001$), and interactions between plant extracts and concentrations (F = 9.77, d.f. 21, 112, $p < 0.0001$), between concentrations and observation times (F = 3.52, d.f. = 6, 224, $p = 0.0046$), and among plant extracts, concentrations and observation times (F = 1.53, d.f. = 42, 224, $p = 0.0392$). The interaction between plant extracts and observation times was not significant ($p > 0.05$). Except for the celangulin treatment, Abbott-corrected percent mortality increased with observation times and concentrations in all plant extract treatments (Table 4). Veratrine achieved >45% mortality, and cnidium lactone and matrine achieved >50% mortality only at the concentration of 0.5% and after 72 h of exposure. Stemonine achieved 78% mortality at 0.5% after 72 h, whereas nicotine achieved 76% mortality at 0.1% after 48 h. Pyrethrins and rotenone achieved 100% mortality at 0.1% and 0.5%, respectively, after 48 h.

Table 4. Mean Abbott-corrected percent mortality (±SEM) of western flower thrips adults on periwinkle flowers dipped in plant extract and Tween 20 solutions in the fresh residue toxicity bioassays.

Active Ingredient	Concentration (%)	Mean Percent Mortality [1] at		
		24 h	48 h	72 h
Celangulin	0.01	7.5 ± 4.8	7.5 ± 4.8 b	15.6 ± 9.7
	0.05	5.0 ± 5.0	2.8 ± 2.8 b	13.1 ± 9.7
	0.1	15.0 ± 5.0	20.0 a	15.8 ± 9.2
	0.5	0	0 b	16.3 ± 3.8
Cnidium lactone	0.01	6.0 b	12.0 ± 3.7	12.4 ± 4.0 b
	0.05	14.0 ± 2.4 ab	14.7 ± 2.7	27.6 ± 8.9 ab
	0.1	18.0 ± 3.7 ab	18.9 ± 6.1	34.9 ± 7.5 ab
	0.5	27.3 ± 9.0 a	33.1 ± 12.8	50.2 ± 10.4 a
Matrine	0.01	5.0 ± 2.9 b	5.0 ± 2.9 b	7.8 ± 2.6 c
	0.05	8.0 ± 2.0 b	8.0 ± 3.5 b	18.4 ± 3.5 bc
	0.1	10.0 ± 3.2 b	15.1 ± 2.9 b	24.0 ± 4.2 b
	0.5	36.7 ± 11.8 a	41.3 ± 10.8 a	51.1 ± 7.4 a
Nicotine	0.01	12.4 ± 4.0 d	18.4 ± 6.7 d	28.0 ± 9.9 c
	0.05	48.0 ± 4.9 c	58.2 ± 4.9 c	66.7 ± 1.8 b
	0.1	70.0 ± 5.5 b	76.4 ± 6.4 b	78.4 ± 4.6 b
	0.5	93.6 ± 4.4 a	100 a	100 a
Pyrethrins	0.01	55.1 ± 6.7 b	63.3 ± 4.9 b	66.9 ± 5.6 b
	0.05	92.0 ± 3.7 a	93.8 ± 4.1 a	97.8 ± 2.2 a
	0.1	98.0 ± 2.0 a	100 a	100 a
	0.5	100 a	100 a	100 a
Rotenone	0.01	18.0 ± 8.6 c	20.0 ± 8.4 c	24.7 ± 6.6 d
	0.05	36.0 ± 10.3 bc	44.2 ± 9.7 bc	50.2 ± 7.6 c
	0.1	52.0 ± 9.7 b	63.1 ± 10.1 b	74.6 ± 10.4 b
	0.5	93.8 ± 2.5 a	100 a	100 a
Stemonine	0.01	3.3 ± 3.3 b	3.3 ± 3.3 b	13.3 ± 3.3 b
	0.05	7.5 ± 4.8 b	7.5 ± 4.8 b	15.0 ± 6.5 b
	0.1	16.0 ± 4.0 b	17.3 ± 4.6 b	26.0 ± 7.4 b
	0.5	35.6 ± 7.3 a	59.1 ± 8.0 a	77.9 ± 3.1 a
Veratrine	0.01	0	0	3.3 ± 3.3
	0.05	2.5 ± 2.5	10.3 ± 4.0	12.9 ± 8.8
	0.1	4.0 ± 2.4	6.0 ± 4.0	12.9 ± 8.8
	0.5	24.4 ± 7.3	27.8 ± 8.2	47.9 ± 10.9

[1] Arcsine-transformed Abbott-correct mortality rates were analyzed with repeated measure ANOVA at α. α = 0.05. Means followed by the same letters within the same active ingredient and observation time combination (in a column) are not significantly different among concentrations by Fisher's least significant difference test at α = 0.05.

3.3. Aged Residue Toxicity

Flowers sprayed with Tween 20 solution and aged in a greenhouse caused 0% to 8.3% mortality at all observation times. Abbott-corrected percent mortality in the aged residue toxicity bioassay differed significantly among plant extracts ($F = 7.06$, d.f. = 3, 61, $p = 0.0004$), residual ages ($F = 23.58$, d.f. = 3, 61, $p < 0.0001$) and observation times ($F = 21.93$, d.f. = 2, 122, $p < 0.0001$). The interaction between plant extracts and residual ages was significant ($F = 4.15$, d.f. = 6, 122, $p = 0.0018$) but other two- and three-way interactions among plant extracts, residual ages and observation times were not significant ($p > 0.05$). Only 1-d-old residue of pyrethrins, nicotine and rotenone achieved significantly higher Abbott-corrected percent mortality in WFT cohorts than older residues (Table 5). For these active ingredients, the highest mortality was <26%. Stemonine appeared to have greater residual toxicity than other active ingredients, with 1-d-old residue achieved >30% mortality and 5-d-old residue maintained >15% mortality at 48 h after exposure.

Table 5. Mean Abbott-corrected percent mortality (± SEM) of western flower thrips adults exposed to periwinkle flowers with residue of various ages after the plants were sprayed with various plant extract and Tween 20 solutions in the aged residue toxicity bioassays.

Active Ingredient	Residual Age (Days)	Mean Percent Mortality [1] at		
		24 h	48 h	72 h
Nicotine (at 0.1%)	1	15.1 ± 3.5 a	22.2 ± 6.9 a	25.9 ± 5.9 a
	3	4.0 ± 0.2 b	4.0 ± 0.2 b	6.0 ± 0.2 b
	5	0 b	10.1 ± 5.8 ab	10.1 ± 5.8 b
	7	2.2 ± 2.2 b	2.2 ± 2.2 b	6.2 ± 2.6 b
Pyrethrins (at 0.05%)	1	18.5 ± 4.1 a	21.9 ± 4.1 a	22.1 ± 4.0 a
	3	2.0 ± 2.0 b	2.0 ± 2.0 b	2.0 ± 2.0 b
	5	0 b	0 b	0 b
	7	2.5 ± 2.5 b	2.5 ± 2.5 b	2.5 ± 2.5 b
Rotenone (at 0.1%)	1	18.5 ± 4.1 a	20.2 ± 3.7 a	24.0 ± 3.3 a
	3	2.5 ± 2.5 b	2.5 ± 2.5 b	2.5 ± 2.5 b
	5	0 b	2.5 ± 2.5 b	5.0 ± 5.0 b
	7	2.5 ± 2.5 b	2.5 ± 2.5 b	7.5 ± 2.5 b
Stemonine (at 0.5%)	1	13.4 ± 2.1	28.8 ± 3.2 a	32.7 ± 6.8
	3	10.0 ± 2.6	20.9 ± 5.9 a	24.4 ± 6.8
	5	10.0 ± 4.1	15.4 ± 2.8 ab	17.7 ± 7.6
	7	6.7 ± 3.4	6.7 ± 3.4 b	15.4 ± 5.1

[1] Arcsine-transformed Abbott-correct mortality rates were analyzed with two-factor repeated measure ANOVA at $\alpha = 0.05$. Means followed by the same letters within the same active ingredient and observation time combination (in a column) are not significantly different among residue ages by Fisher's least significant difference test at $\alpha = 0.05$.

Plants treated with Tween 20 solution did not develop any phytotoxicity on flowers or leaves at any time during this experiment. All plant extract treatments resulted in some levels of bleaching of all the flowers. For the affected flowers, >40% of flower surfaces were bleached (which is typically considered unacceptable in the floricultural trade) within 3 h of application. The severity of phytotoxicity did not increase during subsequent daily evaluations, suggesting that the flowers were damaged on contact with the plant extract solutions. The plant extract solutions, however, did not result in any observable phytotoxicity on the leaves of the treated plants. Plant extract containing stemonine left a layer of black residue on all leaf surfaces of the treated plants, whereas no residue was observed on plants treated with other plant extracts.

4. Discussion

Plant secondary metabolites represent a rich source of medicinal and pest management compounds. Several plant extracts tested in this study contain compounds that have demonstrated antioxidant, antimicrobial, anticancer and other medicinal properties [16–18]. Some have been tested for their insecticidal and acaricidal properties, whereas others (e.g., matrine, nicotine, pyrethrins and rotenone) were previously or are still available as formulated insecticides or acaricides.

Celangulin is extracted from the Chinese bittersweet, *C. angulatus*, which has traditionally been used as an insecticidal plant in China [19]. Proposed modes of action of celangulin include disruptions of normal membrane potential in midgut and signaling pathway in neuron [20–26]. The majority of studies have examined the efficacy of celangulin against lepidopteran larvae [21–26] and coleopteran adults [27]. This study represents the first attempt to evaluate the insecticidal activity of celangulin against WFT. Our results suggested that celangulin did not provide repellency against WFT at the concentration of 0.05% and did not provide sufficient contact toxicity against WFT at concentrations ranging from 0.01 to 0.5%. The maximum percent mortality was <20% when WFT were in contact with fresh residue. Formulated products containing 0.15% to 0.2% celangulin have purportedly been

developed [19]. Our study indicated that celangulin formulated at these concentrations is not likely to be effective against WFT.

Veratrine has been documented as a voltage-gated sodium channel activator in rat [28] and a dose-dependent, non-competitive inhibitor of acetylcholinesterase in Drosophila [29]. The insecticidal activity of sabadilla seed powder has been known since the 16th century [30]. Veratrine, the most toxic of sabadilla alkaloids [30], has being studied as an insecticide in the 1940s [31–33]. Since then, few scientific evaluations of the efficacy of veratrine as an insecticide have been published. Devitt et al. [34] reported that all *Euxoa messoria* (Harris) (Lepidoptera: Noctuidae) first instars died within a week of being offered diet containing veratrine. Although Weinzeirl [35] and Thacker [36] have indicated the use of veratrine for thrips management, to our knowledge, this study is the first detailed evaluation of the repellency and toxicity of veratrine against WFT. Our results indicated that veratrine did not provide significant repellency at 0.05%. Fresh residue of veratrine also did not provide quick knockdown of WFT population, with the concentration of 0.5% achieving only 48% mortality after 72 h of exposure. Future insecticidal products should contain more than 0.5% veratrine in the mixed solution when it is used against WFT.

Stemonine was the only novel plant compounds of interest to us that had demonstrated some level of repellency and toxicity against WFT. *Stemona* spp., which contain more than 80 alkaloids, have a long history as medicinal and insecticidal plants in Asia [37,38]. The majority of insecticidal products were extracted from the roots of *Stemona* plants, demonstrating neurological, antifeedant and growth regulating activities [37,38]. Venkateshalu et al. [39] reported that Stanza, a botanical insecticide that contains extracts of *Stemona tuberosa* Lour. root and pink plume poppy, reduced chilli thrips (*Scirtothrips dorsalis* Hood) densities, but the active insecticidal alkaloid was not identified. In this study, plant extract containing stemonine demonstrated repellency at 0.05% (seconded to that of pyrethrins) in the two-choice (plant extract) bioassays but failed to repel WFT even at the concentration of 0.1% in the two-choice (concentrations) bioassays. It is not clear why the results from the two bioassays suggested different repellencies. Fresh 0.5% stemonine residue achieved 78% mortality at 72 h after exposure, but 1-d-old residue from 0.5% stemonine only achieved 33% mortality within the same exposure period. The comparison suggests rapid breakdown of stemonine residue under sunlight. Despite this rapid degradation, stemonine still achieved greater aged residual toxicity than other plant extracts where 7-d-old stemonine residue was the only one achieving 15% mortality after 72 h of exposure (<8% for other extracts). The inconsistency in the repellency and toxicity of stemonine warrants additional studies to evaluate its potential as a botanical insecticide. We suggest that future evaluations of the efficacy of stemonine against WFT should focus on identifying effective concentrations above 0.5%.

Matrine and oxymetrine have been developed into commercial insecticides, and several studies had demonstrated their efficacies against various insect pests. Matrine applied at 0.5 to 1.5 mL/L achieved similar mortality and banana fruit damage by *Thrips hawaiiensis* Morgan as abamectin [40]. Oxymatrine or matrine had LC_{50} values against adult and immature WFT that were comparable to those of fipronil and much higher than those of cypermethrin, diazinon and imidacloprid [12]. In our study, however, matrine was not effective against WFT. Matrine did not provide repellency against WFT in two-choice bioassays, and direct contact with fresh matrine residue resulted in 51% mortality after 72 h of exposure. Data from this study suggested that formulated product should require application at >0.5% matrine to achieve some level of reduction in WFT abundance and damage.

The goal of our project was to identify plant extracts and active ingredients that can be further developed into effective insecticides or acaricides. Our results indicated that plant extracts containing celangulin, cnidium lactone, matrine and veratrine did not provide repellency and toxicity against WFT at a level generally considered acceptable or effective. Stemonine demonstrated repellency and toxicity, albeit at lower levels than nicotine, pyrethrins and rotenone. The efficacy of stemonine may be improved with higher application concentration (>0.5%) or frequency. Its crop safety (i.e., in reducing

phytotoxicity) and residual longevity needs to be improved with more refinement in extraction solvent and methods. Additional research will be needed to further evaluate the potential of stemonine as a botanical insecticide against thrips.

Nicotine, pyrethrins and rotenone were the most effective products against WFT in this study, an observation that is supported by previous research on WFT and other thrips species e.g., [11,41–44]. Pyrethrins and pyrethroids hyperactivate voltage-gated sodium channels, leading to paralysis and death of insects [45]. Additionally, pyrethrins also achieve repellency against insects through activation of olfactory receptor neurons [46]. The dual mode of activity, i.e., toxicity and repellency, makes pyrethrins a valuable tool in preventing infestation and damage and reducing pest abundance. Commercial products containing pyrethrins are currently widely available for management of insect pests in various commodities, use sites and countries, allowing easy access of this valuable pest management tool by many farmers.

Rotenone and nicotine were the second and third most effective compounds in this study, respectively. Rotenone inhibits normal functions of complex I of the mitochondrial respiratory chain and stimulates the production of reactive oxygen species, leading to limitation of adenosine triphosphate (ATP) synthesis and damage of mitochondria (and thus apoptosis), respectively [47,48]. Nicotine and neonicotinoids activate nicotinic acetylcholine receptor subunits, leading to hyperactivity and eventually death of insects [49]. Although products containing nicotine and rotenone are largely unavailable or their pest management uses greatly limited in the USA, Europe, and other developed countries, nicotine and rotenone still represent effective, widely available, and economical pest management options in many developing countries. As demonstrated by this study, nicotine and rotenone are effective and viable botanical insecticides against WFT, particularly in countries where the use of these products is not restricted, or the availability of other effective products is limited. Due to their high mammalian toxicities, nicotine and rotenone must be used carefully to limit risks and impacts to human, non-target animals and the environment.

We observed unacceptable levels of phytotoxicity on plants grown in greenhouse and sprayed with 0.1% nicotine, 0.05% pyrethrins, 0.1% rotenone and 0.5% stemonine. The damage appeared on the more tender flower tissues, instead of on hardened leaf tissues. In the laboratory, such phytotoxicity did not appear on flowers that were dipped in solutions of the extraction solution (ethanol), adjuvant (Tween 20) and plant extracts, suggesting that the cause of the phytotoxicity was not likely the chemicals used in this study. The appearance of phytotoxic reactions under sunlight (greenhouse) but not under artificial light (laboratory) suggested that the chemicals in the plant extract products might have interacted with ultraviolet light or other environmental factors to damage the flowers. Future insecticidal products containing pyrethrins, nicotine, rotenone, and stemonine will need to be formulated carefully to avoid such phytotoxicity. It would also be advisable to use the products for managing pests on leaves, instead of flowers or other tender tissues, to avoid phytotoxicity.

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