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Determination of Lethal Concentrations and Lethal Times of Extracts from *Tanacetum cinerariaiaefolium*, *Derris elliptica*, and *Sophora flavescens*, to Control Green Peach Aphid, *Myzus persicae*

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복숭아혹진딧물, *Myzus persicae*, 방제를 위한 제충국, 데리스, 고삼 추출물의 살충농도와 살충시간 결정

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ABSTRACT: Botanical extracts are employed in management of aphids. Extracts from *Tanacetum cinerariaiaefolium*, *Derris elliptica*, and *Sophora flavescens* are widely used to control various insects. In this study, we determined concentrations of insecticidal active ingredients in commercial botanical extracts of these plants, and we investigated the time and concentration for lethal results with the green peach aphid, *Myzus persicae*. The concentrations of active ingredients, pyrethrins from *T. cinerariaiaefolium*, rotenone from *D. elliptica*, and matrine and oxymatrine from *S. flavescens*, were determined after their fractionation by liquid chromatography followed by mass analysis and comparison with standard compounds. The extracts were tested for lethality in a bioassay with green peach aphids. Sprays at defined doses were applied to tobacco leaves infested with aphid nymphs. The lethal concentrations (LC50) were 20.4 ppm for pyrethrins, 34.1 ppm for rotenone, and 29.6 ppm for matrine at 48 h after treatments. At 100 ppm application levels, the lethal time LT50 was 13.4 h for pyrethrin, 15.1 h for rotenone, and 14.4 h for matrine. Kaplan-Meier analysis indicated the lethal times for the three botanical extracts at 100 ppm were significantly faster than application of a chemical insecticide, Sulfoxaflor, applied at the recommended level. These results provide baselines to develop and formulate single or mixed preparations containing botanical extracts to control green peach aphids on commercial crops.

Key words: Green peach aphid, Probit, Pyrethrins, Matrine and oxymatrine, Rotenone

초 록: 제충국(*Tanacetum cinerariaiaefolium*), 데리스(*Derris elliptica*), 고삼(*Sophora flavescens*) 추출물은 다양한 해충을 방제하는데 사용되고 있다. 하지만, 국내에서 판매되고 있는 식물추출물 자재는 유효성분의 표기가 없고, 살충농도와 살충시간에 대한 자료가 전무한 상황이다. 본 연구에서는 상용화된 주요 식물추출물의 살충유효성분의 농도를 결정하고 복숭아혹진딧물에 대해 살충농도와 살충시간을 측정하였다. 식물추출물의 살충활성성분인 pyrethrins, rotenone, matrine과 oxymatrine의 농도는 액체 크로마토그래피에서 표준물질을 활용하여 질량분석을 통해 측정하였다. 식물추출물을 농도별로 희석하여 복숭아혹진딧물에 살포하여 살충력을 측정하였다. 표준화합물과 비교한 후 질량분석 및 결정했습니다. *Myzus persicae*에 대한 lethal concentation과 lethal time을 조사했다. 살포 후 48시간 후 치사 농도(LC50)는 pyrethrins (20.4 ppm), roteone (34.1 ppm), matrine (29.6 ppm)였고, 100 ppm 살포한 LT50은 pyrethrins (13.4시간), rotenone (15.1시간), matrine (14.4시간)로 측정되었다. Kaplan-Meier 생존분석 결과, 100 ppm에서 세 가지 식물 추출물의 LT50은 대조구인 화학 살충제인 Sulfoxaflor를 살포 처리구보다 유의하게 빨랐습니다. 본 결과는 복숭아혹진딧물 방제를 위해 식물추출물의 제형화에 단일 또는 혼합 제제를 개발하는데 기준 살충농도와 살충시간을 제고하는데 의미가 있다.

검색어: 복숭아혹진딧물, probit 분석, 피레쓰린, 마트린, 로테논

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Many plant extracts contain compounds that have insecticidal activity. Interest in their commercialization in insecticidal formulation exists in part because of increasing development of resistance to current chemical synthetic insecticides (Silva et al., 2012; Bass et al., 2014; Kim, 2021). Additionally, botanically-derived compounds may have lower mammalian toxicity and persist for less time in the environment making them safer products for insecticide use. Natural pyrethrum, extracted from the flowers of *Tanacetum cinerariifolium* (Trevir.), an aster known as the pyrethrum daisy, has the longest history of use (Pavela, 2016) and holds 80% of the total market of global botanical insecticides (Isman, 2005). Industrial production of the pyrethrin insecticides currently requires large-scale cultivation of this plant, also known as *Dalmatium pyrethrum* in Europe. Pyrethrins accumulate to 1-2% of dry mass in the mature flower heads, which are harvested, dried, and powdered (Chen et al., 2018; Grdiša et al., 2022). They have a wide insecticidal range that includes spider mites, flies, fleas and beetles (Casida and Quistad, 1998; Rattan, 2010). Pyrethrins do not readily spread within plants so that their activities are direct with the target insect and are without systemic effects (Isman, 2006; Rattan, 2010; Lengai et al., 2020).

In the extracts from *T. cinerariifolium* insecticidal activity is correlated with several metabolites, termed pyrethrin I and II, jasmolin I and II and cinerin I and II (Chen et al., 2018). The structures of these Ais are shown in Fig. 1A and illustrate that the compounds designated as II forms bear an extra acid group compared with compounds destinated as I. Blockage of voltage-gated sodium channels in insect nerve axons is correlated with the activity of the pyrethrins (Soderlund, 2012).

Although they cause immediate knockdown of aphid populations rapid degradation of pyrethrins can require frequent application for control efficacy (Lengai et al., 2020). Their limited stability also is an advantage for nontarget effects; pyrethrin I and II (Isman, 2006) are toxic to bees and fishes (Johnson et al., 2006; Ingram et al., 2015). Their breakdown is promoted by sunlight limiting persistence in water, soil and on plant surfaces. Half-lives for pyrethrin I are 11.8 h in water and 12.9 h on soil surfaces. Pyrethrins do not dissolve well in water but bind strongly to sediment. Half-lives of pyrethrin 1 in sediment are 10.5 to 86 d (Wauchope et al., 1992; Antonious et al., 2001; Angioni et al., 2005). On potato and tomato leaves, less than 3% remained after 5 d (Antonious et al., 2001; Angioni et al., 2005). In the absence of light, pyrethrin 1 breaks down more slowly in water. Half-lives of 14 to 17 days have been reported in water with delayed times with acidity.

Different molecular targets are found with other insecticidal botanical products. Inhibition of ATP synthesis correlates with the insecticidal activity of rotenone cited as the active insecticide in extracts from the roots of *Derris elliptica* (Haller et al., 1942; Lawrence, 1956; Rattanapan, 2009). However, there are nontarget effects. Because of its toxicity to fish, rotenone is used to manage fish populations (Krumholz, 1948; Robertson and Smith-Vaniz, 2008; Melo et al., 2015) meaning that run off into streams could be a problem. The alkaloids matrine and oxymatrine, which are extract from the roots and seeds of *S. flavescens* poison the gastrointestinal activities in the insect as well as acting on the nervous system (He et al., 2015; Xiong et al., 2016). Many insects in addition to mites such as cut and wireworms and maggots are sensitive to the *S. flavescens* products. Interestingly, matrine acts synergistically with the insect entomopathogenic fungus *Beauvaria* suggesting that compound formulations could be very effective (Wu et al., 2019).

In this study, we determined whether the anticipated insecticidal compounds were present in commercial extracts from *C. cineariaefolium, D. elliptica, S. falvescens* and quantified their concentrations using LC-MS/MS analysis. We compared the efficacy of these commercial extracts on the green peach aphid. This aphid *Myzus persicae* (Sulzer), is a hazard for crops (Capinera, 2001) because not only does it weaken the plant through utilization of plant sap for its reproduction but it transmits viral diseases (Ng and Perry, 2004) and can incite onset of sooty mold through the aphid's secretion of honeydew (Capinera, 2001). The insecticidal activity was determined using a bioassay developed for reproducibility based on feeding and reproduction of the aphid's nymphs on non-infested tobacco leaves (Cho et al., 2023). The findings were compared with the efficacy of a current commercial insecticide with broad usage, Sulfoxaflor, which is classified as a sulfoximine (Sparks et al., 2013). This product is a systemic insecticide acting as an nicotic actylcholine receptor (nAChR) competitive modulator (Casida and Durkin, 2013). The product acts through contact or ingestion. Although of a different structure than any of the botanical

products tested, it impairs neuronal function in insects (Sparks et al., 2013; Li et al., 2021). Our recent bioassays on green peach aphid indicated Sulfoxaflor exhibited LT50 29.6 h and LT90 54 h with standard recommended dose application, approximately 25 ppm (Cho et al., 2023).

The chemical characterization of the botanical extracts indicates that presence of the anticipated insecticidal compounds. Lethal concentrations and times for the botanical extracts and Sulfoxaflor were determined from dosed studies. The bioassay showed that these had dose - dependent toxicity for the green peach aphid. These results indicate that extracts from the plants can be generated commercially that have potential for their use in agricultural formulations for aphid control.

Materials and Methods

Green peach aphid breeding

Green peach aphids (*Myzus persicae*) were provided by Dr. Duck Soo Choi from the Jeonnam Agricultural Research and Extension Services (Naju, South Korea). Aphids were maintained on tobacco (*Nicotiana tabacum* L. 'Xanthi') seedlings grown for 4 weeks with a photoperiod of 16 h light: 8 h dark under 40 W fluorescent lights (2000 lux, 80 μmol photons/m^{-2} s^{-1}). The temperature was maintained at 21 ± 2°C with a relative humidity of 50-60%. To obtain nymphs, healthy adult apterous aphids were transferred to non-infested 4 weeks-old tobacco leaves on intact plants using 20 adults/leaf. These tobacco plants were placed into cages for 5 days while they produced the third- or fourth instar nymphs used in the bioassays.

Liquid-Chromatography-Mass Spectrometry/MS analysis

Concentrations of compounds with authenticated insecticidal effects in commercial liquid extracts of *T. cinerariaiaefolium*, *D. elliptica*, and *S. flavescens* were determined using LC-MS/MS analyses. The extracts were purchased from Greenfocus Com., (Goyang Si, Gyeonggi-do, Korea). According to company's procedure, the commercial preparations were of pyrethrins from dried flowers of *T. cinerariaiaefolium*, rotenone from dried roots of *D. elliptica*, and matrine from dried roots of *S. flavescens.* The compounds were extracted from dried powders into ethanol at room temperature for 3 weeks. After we purchased the liquid botanical extracts, the suspensions were filtered through 0.2 μm filters (Whatman, PTFE) before storage at room temperature under dark conditions in brown glass bottles, and diluted to 1,000-fold with methanol and used for LC-MS/MS analysis. The LC-MS/MS analyses were performed with an AB SCIEX Qtrap4500 QTRAP with the Waters AQUITY UPLC H-Class (Framingham, MA, USA) instrument. A Cardenza, CD-C18 UP, 2.0 × 150 mm, 3.7 μm column (Imtakt Com., Portland, OR, USA) was used with separation solvent A (5 mM ammonium acetate + 0.1% (v/v) formic acid in water) and B (5 mM ammonium acetate + 0.1% (v/v) formic acid in methanol). The injection volume was 2 μl and the flow rate was 0.2 ml/min for gradient elution for 7 min. All compounds were ionized by electrospray ionization (ESI) in positive mode and were analyzed by multiple reaction monitoring (MRM).

The reference standards that have documented insecticidal functions were purchased for the MS analysis. Compounds from Sigma-Aldrich (St. Louis, MO, USA) were matrine (CAS number 519-02-8), oxymatrine (CAS number 16837-52-8), and rotenone (CAS Number 83-79-4). Other authentic compounds were from AccuStandard (New Haven, CT, USA): pyrethins I (CAS Number 121-21-1) and II (CAS Number 121-29-9), cinerin I (CAS Number 23402-06-6) and II (CAS Number 121-20-0), and jasmolin I (CAS Number 4466-14-2) and II (CAS Number 1172-63-0). Each standard solution was prepared at 50 ppm in methanol and was serially diluted before injection to conduct LC-MS/MS analysis as described above. Standard curves were generated from the outputs. These calibration curves were established, immediately before assays of the test samples, so that the concentration of metabolites in the botanical extracts could be calculated. Three independent experiments were conducted with three replicates per each experiment.

Aphicidal bioassays

Extracts from *C. cinerariaiaefolium*, *D. elliptica*, and *S. flavescens* were diluted to be 200, 100, 25, 12.5 ppm with sterile water to yield preparations that were assessed using a bioassay based on a recently developed leaf infestation method

(Cho et al., 2023). Sprays of 2,000 x diluted commercial Strait™ (7% Sulfoxaflor) were used as a positive control (Dongbang Agro Co. Seoul, Korea) to generate was used at 25 ppm as suggested by the manufacturer. Sterile water was sprayed as the negative control.

The bioassay followed methods of Cho et al. (2023). Briefly, water agar (Junsei, Japan), in insect breeding dishes (5.0 x 1.5 cm, SPL, Korea) was overlayed with 5.0 cm circles cut from 4 weeks-old non-infested tobacco leaves. Twenty third or four instar nymphs (approximately >1mm in length) were placed onto each tobacco leaf using a soft brush. Each plate was sprayed with 1 ml of the potential insecticide solution from a 20 cm vertical distance using mini 2 ml-capacity glass bottle atomizers (5.35 x 3.62 x 3.19 inches; Brand Csdtylh 0167, Amazon, USA). The breeding dishes were incubated under a 14 h light/10 h cycle under 40W fluorescent lights (70 μmol photons/m^{-2} s^{-1}) to allow nymph development into adults. All stages of the aphid that occur in this bioassay were feeding on the host tobacco leaf. The temperature was maintained at 21 ± 2°C with a relative humidity of 60% for 3 d. The viability of the insects on the leaf surface was assessed under a stereoscopic microscope (Model C-LEDS, Nikon Imaging Japan Inc., Japan) by touching with a fine brush at defined times and classifying insect bodies that showed no movement as being dead. Three replicates were included for each treatment and the assays were repeated three times.

Because the bioassay process involves some level of mortality to the nymphs even with control sprays of water, a corrected mortality value was calculated according to Abbott's formula (T-C/100-C) x 100, where T is mortality in treatment and C is mortality observed in the control treatment, a water spray (Abbott, 1925). The median lethal times (LT50 and LT90) were calculated using a complementary log-log model (Finney and Stevens, 1948).

Data analysis

Data from the counts of dead insects were analyzed by ANOVA ($P < 0.05$), using XLSTAT LIFE SCIENCES (version 2023.1.4, Addinsoft, Paris, France). If the F test showed significant differences, the differences between measurements were further elucidated through Duncan's multiple range test ($P < 0.05$). The LT50 and LT90 data between methods were analyzed using the Tukey post-test if the variance analysis results were significant at 95% confidence level, using SPSS (IBM Corp., Armonk, NY, USA). The LT50 and LT90 were also examined by using Probit analysis from XLSTAT LIFE SCIENCES. The extent of survival was evaluated with Kaplan-Meier Survival Analysis Log-Rank using XLSTAT LIFE SCIENCES.

Results and Discussion

Concentration of active insecticidal compounds in the commercial botanical extracts

Six pyrethrin isomers were detected in commercial botanical liquid extracts of *T. cinerariaefolium* by LC-MS/MS analysis (Supplemental Fig. 1 and Supplemental Table 1). Major compounds (90%) were pyrethrin I and II, and with lesser levels of jasmolin I and II (6%), and cinerin I and II (3%). The higher amounts of pyrethrin I and II detected agreed with other published analyses of pyrethrum extracts (Casida and Quistad, 1995; Grdiša et al., 2022). Total pyrethrin concentration was 20,302 ppm in the original liquid extract obtained as a commercial *T. cinerariaefolium* extract (Table 1). The commercial *S. flavescens* liquid extract had no detectable level of oxymatrine (Supplemental Fig. 2), but matrine (9,439 ppm) was present (Table 1). Rotenone was detected in the commercial

Table 1. Quantification of major active insecticide ingredients in the commercial liquid botanical extracts

Active ingredients (ppm ± SE) *		
Total Pyrethrins	Rotenone	Matrine
20,303 ± 264	34,080 ± 1,209	9,440 ± 270

*Concentrations of defined insecticidal chemicals in commercial liquid extracts from *T. cinerariaefolium*, *D. elliptica*, and *S. flavescens* as determined by LC-MS/MS analyses (Supplemental Fig. 1, 2, and 3). Data (ppm) are the means and standard errors of three independent measurements.

D. elliptica liquid extract at 34,040 ppm (Supplemental Fig. 3 and Table 1).

Bioassays of the lethal effects of the commercial botanical extracts on the green peach aphid

Each extract showed a lethal effect when sprayed as an aqueous solution onto green peach aphid nymphs feeding on tobacco leaves. The commercial product Sulfoxaflor used at 25 ppm also was toxic to the nymphs. The lethal effects showed dose and time dependency as illustrated for the pyrethrins (Fig. 1), for rotenone (Fig. 2) and for matrine (Fig. 3). Notable is a statistical increase in nymph mortality after 3 h at higher doses with the botanical compounds, unlike the response of the aphids to the systemic insecticide Sulfoaxflor (Fig. 1, 2, and 3).

When the data sets were analyzed for lethal concentrations (LC50/90), the results from each botanical extract showed high regression coefficients throughout ($R>0.93$) (Table 2). The pyrethrin mixture was the most potent especially at the lowest doses. Regression coefficients were all above 0.91 when lethal times (LT50/90) were deduced (Table 3). Each botanical extract showed dose dependent effects on lethality time (Fig. 1, 2, 3 and Table 3). For up to 12 h the efficacy was higher for the

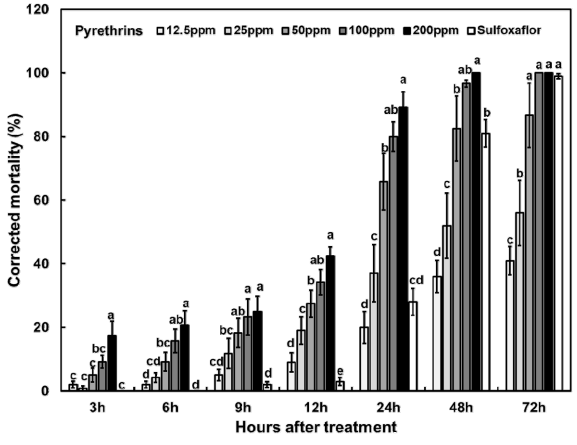

Fig. 1. Corrected mortality of green peach aphids by time period after treatment of defined concentrations in the pyrethrins of *T. cinerariaiaefolium* commercial liquid extract and Sulfoxaflor. Data are the means and standard errors from three independent experiments each with three replicates of the treatments for total pyrethrins in the botanical extract. One-way analysis of variance (ANOVA; $P < 0.05$ was considered significant) was performed using IBM SPSS software version 23 (IBM Corp., Armonk, NY, USA); Duncan's multiple range test ($P < 0.05$) further compared differences if the F test was significant. The lowercase letters indicate statistical differences. Abbott's corrected mortality (%) = (mortality of treatment-mortality of control) / (100-mortality of control) x 100 was used in the pyrethrin treatment.

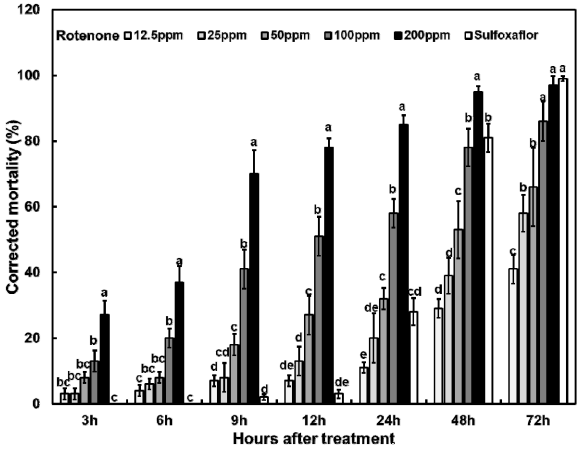

Fig. 2. Corrected mortality of green peach aphids by time period after treatment rotenone from commercial liquid extract of *D. elliptica* and Sulfoxaflor. Data represent three independent experiments means and standard error with three replicates each. One-way analysis of variance (ANOVA; $P < 0.05$ was considered significant) analyzed data using IBM SPSS software version 23 (IBM Corp., Armonk, NY, USA); Duncan's multiple range test ($P < 0.05$) further compared differences if the F test was significant, and lowercase letters indicate statistical differences. Abbott's corrected mortality (%) = (mortality of treatment-mortality of control) / (100-mortality of control) x 100 was used in the pyrethrin treatment.

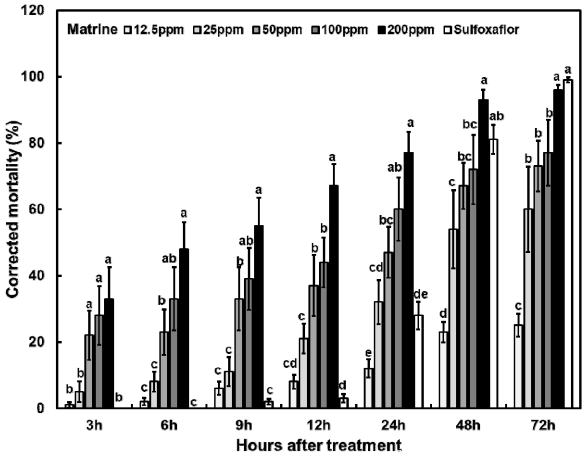

Fig. 3. Corrected mortality of green peach aphids by time period after treatment by matrine contained in the commercial *S. favescens* liquid extract and Sulfoxaflor. Data represent three independent experiments means and standard error with three replicates each. One-way analysis of variance (ANOVA; $P < 0.05$ was considered significant) analyzed data using IBM SPSS software version 23 (IBM Corp., Armonk, NY, USA); Duncan's multiple range test ($P < 0.05$) further compared differences if the F test was significant, and lowercase letters indicate statistical differences. Abbott's corrected mortality (%) = (mortality of treatment-mortality of control) / (100-mortality of control) x 100 was used in the pyrethrin treatment.

commercial product than Sulfoaxaflor. Whereas Sulfoaxflor showed high corrected mortality of 80% or over at 48 and 72h), this extent of killing occurred also with all the botanical extracts (Fig. 1, 2, 3). Effective LT50s were notable after about 20 h of application with the pyrethrin mixture being the fastest treatment (Table 3) whereas 25 ppm Sulfoxaflor showed an LT50 of 30 h (Cho et al., 2023). The LT50s were 22 h for matrine and 41 h for rotenone (Table 3). The Kaplan - Meier survival analysis confirmed that lethal times of all three botanical extracts at 100 ppm application caused significantly faster lethality ($P<0.001$) than the chemical standard insecticide, Sulfoxamide (Fig. 4A). However, no significant difference in lethal time was found at applications of 50 ppm of the botanical extracts or the chemical insecticide (Fig. 4B).

Table 2. Lethal concentration (mg/L) of based on pyrethrins, rotenone, and matrine in commercial botanical extracts against *Myzus persicae*

Hours after treatment (HAT)	LC50 (ppm, 95% CL)	LC90 (ppm, 95% CL)	Regression line and coefficient
Pyrethrins in *T. cinerariifolium* extract			
24	35.62 (30.05~41.63)	181.11 (140.50~254.92)	y = 1.783x - 2.774 R^2 = 0.9849
48	20.45 (17.47~23.38)	65.28 (54.89~82.02)	y = 2.5811x - 3.376 R^2 = 0.9781
72	18.22 (15.54~20.80)	53.73 (45.51~67.09)	y = 2.3236x - 2.9115 R^2 = 0.9469
Rotenone in *D. elliptica* extract			
24	71.32 (61.30~84.10)	339.54 (252.78~507.17)	y = 1.8739x - 3.4496 R^2 = 0.9692
48	34.12 (28.53~40.16)	189.26 (143.88~275.83)	y = 1.8342x - 2.7906 R^2 = 0.9489
72	20.00 (15.36~24.55)	130.77 (99.57~192.39)	y = 1.714x - 2.2484 R^2 = 0.9550
Matrine in *S. flavescens* extract			
24h	61.98 (51.66~75.29)	447.80 (303.12~788.14)	y = 1.5258x - 2.7427 R^2 = 0.9776
48h	29.56 (12.42~49.32)	190.28 (96.39~1,511.11)	y = 1.6476x - 2.4328 R^2 = 0.9339
72h	25.00 (8.23~42.90)	141.75 (73.62~1,260.37)	y = 1.7899x - 2.5169 R^2 = 0.9264

Regression line: the equation reflects the relationship between the aphicidal activity and the diluted botanical extract treatment. LC50 and LC90, median lethal concentration value, and 95% confidence limit (CL).

Table 3. Lethal time (hours) of the efficacy of pyrethrins, rotenone, matrine in commercial botanical extracts against *Myzus persicae*

Concentration (ppm)	LT50 (h, 95% CL)	LT90 (h, 95% CL)	Regression line and coefficient
Pyrethrins in *T. cinerariifolium* extract			
200	12.21 (8.53~17.95)	34.49 (22.24~88.10)	y = 2.819x - 2.9774 R^2 = 0.9141
100	13.38 (10.10~18.15)	36.62 (25.26~72.22)	y = 2.7945x - 3.0849 R^2 = 0.9382
50	19.82 (17.72~22.27)	70.89 (58.70~89.45)	y = 2.2294x - 2.8984 R^2 = 0.9765
Rotenone in *D. elliptica* extract			
200	9.60 (8.15~11.14)	60.33 (46.96~83.65)	y = 1.592x - 1.5722 R^2 = 0.9587
100	15.09 (13.00~17.53)	97.56 (73.33~142.98)	y = 1.5938x - 1.8853 R^2 = 0.9721
50	41.37 (33.84~53.12)	346.23 (218.05~662.04)	y = 1.3719x - 2.2255 R^2 = 0.9542
Matrine in *S. flavescens* extract			
200	9.23 (7.40~11.19)	108.42 (74.30~185.16)	y = 1.2014x - 1.157 R^2 = 0.9944
100	14.40 (11.54~17.94)	251.02 (147.34~557.30)	y = 1.029x - 1.1891 R^2 = 0.9817
50	22.57 (18.27~28.78)	352.14 (200.88~809.66)	y = 1.0656x - 1.4402 R^2 = 0.9625

Regression line coefficients reflect the relationship between the aphicidal activity and the concentration of the active ingredient in the commercial extracts for times after application. LT50 and LT90, median lethal time value, and 95% confidence limit (CL)

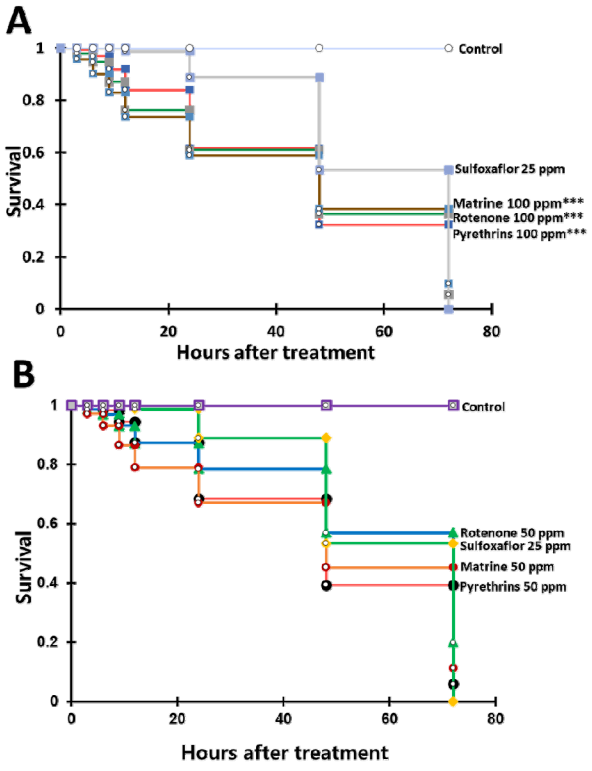

Fig. 4. Kaplan-Meier survival plots of green peach aphids (n = 90) treated with 100 ppm (A) and 50 ppm (B) of pyrethrins, rotenone, matrine from the commercial plant liquid extracts or for both (A) and (B) Sulfoxaflor, 25 ppm. Green peach aphid mortalities were monitored at defined hours post-treatment. Data points represent the average of three experiments with three replicates each. One representative data set is shown. *P* values indicate significant differences between two concentrations in multiple comparisons as determined by the Log-Rank test.

The studies confirmed the presence of anticipated active insecticidal compounds in the three commercial botanical extracts selected for study. This work thus provides a method to confirm the presence of authentic insecticidal compounds and quantify their mass in an extract. The work also demonstrated that quantifiable data can be generated by bioassay against nymphs of an aphid feeding on a defined area of tobacco leaf. On a mass basis, the mix of pyrethrin compounds was more effective than rotenone or matrine. The bioassays with each of the botanical extracts showed fast lethal effects on the nymphs that could relate to direct effects on the insects compared with the longer times needed for lethality of Sulfoxaflor because of its systemic activity. The compounds in each of the botanicals all have nontarget effects that should be considered for field use, but this is also true of the chemically - synthesized insecticides on the market. Limiting run off and timing of application are two methods to minimize nontarget effects. In addition, insect control using botanical extracts may be an alternative to delay development of chemical insecticide-resistant insects. Our LC and LT data are valuable to formulation of botanical extracts to control green peach aphids. We currently are investigating the effects of the botanical extracts on other economically important insect pests, including the diamond-back moth (Plutella xylostella), two spotted spider mite (*Tetranychus urticae*), and yellow tea strips (*Scirotothrips dorsalis*). Additionally formulation with other effective strategies may allow these botanical products to be used at lower doses, thus, lessening risk while maintaining efficacy.

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Supplementary Information

Supplementary data are available at Korean Journal of Applied Entomology online (http://www.entomology2.or.kr).

Statements for Authorship Position & Contribution

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