# Efficacy of essential oil of *Piper aduncum* against nymphs and adults of *Diaphorina citri*

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**Abstract**

BACKGROUND: Insecticide application is the main way to control *Diaphorina citri*. However, it causes environmental contamination, has a negative impact on beneficial organisms, and leads to psyllid resistance. The essential oil of *Piper aduncum* has low toxicity towards the environment and contains dillapiol, which was proven to be effective against several crop pests. Here, we studied its efficacy against nymphs and adults of *D. citri* under laboratory conditions. Oils with three concentrations of dillapiol (65.2%, 76.6%, and 81.6%) at 0.5%, 0.75%, and 1.0% dilutions plus 0.025% adjuvant were tested.

RESULTS: All treatments caused 90–100% mortality in nymphs. Topical treatments with oil containing 76.6% and 81.6% of dillapiol at 0.75% and 1% dilutions were effective (mortality ≥ 80%) in adults. However, the essential oil showed no residual activity against adults (mortality ≤ 30%).

CONCLUSIONS: Dillapiol-rich oil is a promising compound for *D. citri* control.

**Keywords:** Asian citrus psyllid; HLB; botanical insecticide; active ingredient rotation; integrated pest management.

## 1 INTRODUCTION

Pathogens spread by insect vectors are limiting factors for the cultivation of citrus. In particular, the phloem-infecting bacteria *Candidatus* Liberibacter asiaticus and *Ca.* Liberibacter americanus have been associated with the destructive citrus greening disease or Huanglongbing (HLB) that affects commercial citrus varieties on the American and Asian continents.[1–3] The spread of HLB in orchards mainly occurs via the citrus psyllid *Diaphorina citri* Kuwayama that has the ability to transmit both bacterial species.[4,5]

Management of HLB includes the use of citrus trees produced in screened vector-free nurseries, inspection and eradication of diseased plants in orchards, and control of *D. citri* with applications of insecticides.[6] Chemical control, mostly by the active ingredient imidacloprid, is the primary method used for management of the insect vector.[7,8] However, insects have developed resistance against chemicals due to their frequent use, which leads to a greater selective pressure. In Florida, *D. citri* was reported to have a resistance ratio higher than 30 for imidacloprid, followed by chlorpyriphosphos (17.9), thiamethoxam (15.0), malathion (5.4), and fenopropathrin (4.8).[9] The use of different control tactics can slow down the development of resistance and contribute to sustainable use of insecticides in the management of *D. citri*.[8]

One such alternative that requires additional studies is the use of botanical insecticides. Studies on these insecticides for control of *D. citri* are incipient and most of them are focused on the use of extract and essential oil of neem (*Azadirachta indica* A. Juss.). Neem has been demonstrated to have an efficacy against *D. citri* nymphs of 92% in greenhouse and approximately 30% in field conditions.[10,11] Khan et al.[12] reported 80% mortality of adult psyllids in the field using neem extract. Neem and *Datura alba* Nees extracts reduced the number of *D. citri* nymphs and adults by up to 4-fold compared to untreated areas in field conditions.[13]

*Piper aduncum* L., a plant abundant in the Amazon region, displays insecticidal properties[14] because it contains secondary metabolites that show toxicity towards insects, especially monoterpenes,[15] sesquiterpenes,[16] and phenylpropanoids,[17] with the phenylpropanoid dillapiol being the major compound.[18–20]

Dillapiol potentially inhibits the activity of cytochrome P450-dependent monooxygenase, which transform lipophilic compounds intomore soluble (hydrophilic) and easily excretable products.[21] Through inhibition of monooxygenase, the ability of the herbivore insect sto excrete xenobiotics present in the host plant is reduced, resulting in death due to accumulation of toxic substances in its digestive tract.[22,23] Bernard et al.[18] observed a 95% increased mortality of *Ostrinia nubilalis* (Hübner) using dillapiol extracted from *Piper cubeba* L. incorporated in an artificial diet at 100 μg/g. In a study on sucking insects, Silva et al.[24] reported mortalities of 72% and 80% for *Aetalion* sp. adults by using *P. aduncum* extracts from leaves and roots, respectively, both at a concentration of 30 mg $mL^{-1}$. Castro et al.[25] observed 54.8% loss of viability in *Aleurocanthus woglumi* Ashby eggs after topical application of a 4% aqueous *P. aduncum* leaf extract. Previous studies have determined the insecticidal effect of essential oil of *P. aduncum* (OPA) on defoliating pests such as *Cerotoma tingomarianus* Bechyné,[26] flour pest *Tenebrio molitor* L.,[27] and stored-grain pest *Sitophilus zeamais* Motschulsky.[28] However, currently, there are no reports on the toxic activity of *P. aduncum* (dillapiol) towards *D. citri*.

The objective of the present study was to evaluate the efficacy of dillapiol-rich OPA on nymphs and adults of *D. citri* in oursearch for a new mode of action to be adopted in the rotation of active ingredients for controlling this insect vector.

## 2 EXPERIMENTAL METHODS

### 2.1 OPA extraction and quantification of chemical compounds

Three-year-old adult *P. aduncum* plants were collected in the production field of Embrapa Acre, Rio Branco, Acre, Brazil (10° 1′ 21.36″ S, 67° 42′ 31.70″ W) by cutting them at 0.4 m above the ground surface. Plants were harvested every 12 months for essential oil extraction. The leaves and fine stems were separated for processing. The plant mass was subjected to drying to achieve 20–30% of moisture. Essential oil was extracted by steam distillation as described previously [29] with a yield around of 2–2.5%. The essential oil was redistilled through fractional rectification by using a heating mantle up to 150 °C and a 3000-mL flask. The flask was connected to an absorption tower consisting of a single glass column of 50 × 600 mm completely filled with 6–8 mm Raschig rings. The top of the column was connected to a 40 × 300-mm condenser for cooling water circulation to condense the volatiles. The condenser was connected to a fraction collector with a dispensing system under –760-mmHg pressure created by using a vacuum pump.

For the identification and quantification of chemical compounds, the OPA was analyzed using a gas chromatograph (GC) coupled to a mass spectrometer (MS) GCMS-QP2010 Plus (Shimadzu, Japan) equipped with a capillary column (Restek Rxi-5MS, 10 m X 0.10 mm ID X 0.10 μm film thickness). The GC temperature program consisted of start temperature at 40°C, followed by a temperature ramp of 4°C $min^{-1}$ to 190°C, followed by another ramp of 47°C $min^{-1}$ to 250°C, and then held for 1.10 minutes. This gave a total GC run time of 40 minutes. The injector and detector interface temperature were 250°C and the ion source temperature was 200°C; the carrier gas was He (column flow 0.64 mL $min^{-1}$, split ratio 1:500), and the samples were diluted in methanol (injection of 0.5 μL). Mass spectra were recorded at 70 eV, with a mass range from *m/z* 40 to 350. Chemical characterization was performed by comparison of the obtained mass spectra with those available in the GC-MS spectra database from National

Institute of Standards of Technology (NIST), data from the literature, and Kovats retention indices[30]. For the determination of Kovats retention rates, a mixture of linear alkanes ($C_8$ to $C_{20}$) was injected into the chromatograph[31]. Component relative percentages were calculated on the basis of GC-MS peak areas.

### 2.2 Phytotoxicity of the OPA against *Citrus sinensis* and definition of working concentrations

Before selecting the range of OPA concentrations to assess its efficacy against *D. citri* nymphs and adults, we evaluated the phytotoxic effect on sweet orange shoots using three dilutions with four different concentrations. The experiment was carried out at Fundecitrus (Fundo de Defesa da Citricultura), Araraquara, Sao Paulo, Brazil (21° 48′ 32.35″ S and 48° 9′ 50.82″ W) in a greenhouse (1.60 × 7.90 × 6.0 m) under ambient temperature (27.13°C on average temperature) and relative humidity (71.62% on average) during the entire experimental period.

Forty-two nursery trees (1-year-old *Citrus sinensis* (L.) Osbeck var. Valencia grafted on Swingle citrumelo [*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.]) with three 15–18-cm young shoots per grafted tree were selected. The plants were grown in 20-L pots containing substrate (80% *Pinus* sp. bark, 15% vermiculite, and 5% charcoal) (Multiplant Citrus®, Terra do Paraíso, Holambra, São Paulo, Brazil).

For the preparation of the insecticide sprays, OPA with different concentrations of dillapiol (65.2%, 76.6%, and 81.6%) and dillapiol 99.9% were diluted to 0.5%, 0.75%, and 1.0% v $v^{-1}$ in water, with addition of 0.025% of Silwet® adjuvant (polyester copolymer and silicone at 100%) (Momentive, Itatiba, Sao Paulo, Brazil). The sprays were prepared by diluting the adjuvant in water, and then adding the OPA according to the concentrations established for each

treatment. In addition, two control treatments consisting of pure water and water with 0.025% adjuvant were included (Table 1). The young shoots were sprayed to a point just before runoff (7.0 mL) with the aid of a Brudden® S-600 manually operated sprayer (Brudden, Pompéia, São Paulo, Brazil).

Phytotoxicity was visually assessed in the young shoots at 1, 7, and 15 days after application (DAA) and scored as follows: score 0 (no toxicity), asymptomatic plants; score 1 (mild toxicity), plants with up to 1-mm necrotic spots (burning) on the leaves; score 2 (moderate toxicity), plants with 1–3-mm spots on the leaves and branches; and score 3 (high toxicity), plants with necrotic spots larger than 3 mm on the leaves and/or complete necrosis of young shoots. We used a randomized block experimental design with 14 treatments and 9 replications. Each treatment consisted of three nursery citrus trees containing three young shoots each; each shoot was considered a replicate.

For efficacy studies on *D. citri*, doses that caused no or mild phytotoxicity (scores 0 and 1), or induced moderate toxicity (score 2) in up to 30% of the plants were selected. The treatments that resulted in more than 30% of plants with score 2 and highly phytotoxic treatments (score 3) were excluded from these experiments.

### 2.3 Assessment of the efficacy of the OPA against *D. citri*

#### *2.3.1 Insects, plants, and testing conditions*

The insects were obtained from a *D. citri* rearing established at Fundecitrus. The rearing was maintained on *Murraya paniculata* (L.) in a climatized room (temperature of 25 ± 3 °C, photoperiod of 14 h, and relative humidity of 65 ± 10%). To obtain eggs, plants with young shoots were transferred to acrylic cages (20 × 21 × 55 cm) with an anti-aphid screen and exposed

to adults for 7 days. The plants containing eggs were kept in the cages until the emergence of adults. To evaluate the efficacy of the OPA, seedlings of *C. sinensis* var. Caipira, grown in tubes in screened nurseries, were used. All tests were conducted in the laboratory under the same temperature, photoperiod, and relative humidity conditions as described for the rearing of psyllids.

*2.3.2 Assessment of the efficacy of the OPA on* D. citri *nymphs and adults by topical application*

The OPA treatments used in these experiments were selected based on the phytotoxicity test results (subsection 2.2). We used treatment with imidacloprid (Provado® 200 SC, Bayer CropScience AG, Dormagen, Germany) as a positive control (Table 1). To test the efficacy of OPA against nymphs, each seedling with one young shoot was infested with 10 third-instar nymphs with the aid of a soft paintbrush. The infested plants were subjected to the various OPA spray treatments and maintained in a climatized room. To test the efficacy of OPA against adults, 10 insects at 10 days after emergence were confined on each shoot by using sleeve cages that are pervious to spraying and that covered the whole shoot. The shoots were sprayed until product runoff. The same treatments as described for topical application on nymphs were used (Table 1).

The number of dead insects (nymphs or adults) was counted at 1, 3, and 7 DAA. Nymphs and adults were considered dead when they did not present mobility of legs, wings, and antennae. For nymphs, the efficacy of the OPA was tested only for topical application as after outbreak, the nymphs develop on the same branch until the emergence of adults, not justifying the testing of residual contact. For both tests, a completely randomized block design was used and each seedling represented a replicate. Seven plants were used for the experiment with nymphs and 8 for the experiment with adults. Additionally, a 3 × 3 factorial design

(concentrations of dillapiol × dilutions of OPA used) was used for the adult insects to verify if there was an influence of increasing dillapiol/OPA and OPA/dillapiol concentration ratios on insect mortality.

*2.3.3 Assessment of the efficacy of the OPA on adults of* D. citri *by residual contact*

Ten adults at 10 days after emergence were placed on the shoot of each seedling on the dry residue (2 h after spraying), using the same insect confinement method and spray application as described above. The treatments used in this test were those classified as effective in the topical application test (mortality ≥ 80%) (Table 1) as described in subsection 2.3.2. The assessments and experimental design were similar to those of the topical tests.

### 2.3 Data analysis and statistics

The results of the assessment of the phytotoxic effect were expressed as percentages calculated from the scores attributed to the damage in all young shoots per treatment. The number of dead insects for all efficacy tests was expressed as a percentage. All data were expressed as the mean ± standard error of the mean (SE). The data were transformed into arcsine $(x/100)^{0.5}$ prior to analysis to reduce heteroscedasticity and achieve normality. Means were subjected to analysis of variance (ANOVA) with repeated measures over time, and in case of significance, compared by Tukey's test ($P \leq 0.05$). All analyses were performed using the AgroEstat software.[32]

## 3 RESULTS

### 3.1 Chemical constituents of essential oils

The compositions of the four OPAs obtained by fractional distillation used in the present experiments were determined by GC-MS comparing their relative retention times and the mass spectra of the OPAs components from a data library. We injected 3 samples from each OPA fraction to determine the average percentage ± SE for each fraction of oil. Characterized compounds of these oils with their relative percentages are listed in Table 2. A total of 40, 39, 30 and 6 components were identified in OPA 01, OPA 02, OPA 03 and OPA 04 respectively. Six compounds comprising myristicin, *z*-isoelemicin, caryophyllene oxide, globulol, dillapiol and apiol were present in all four OPAs. Dillapiol was the most abundant compound identified from OPAs obtained by fractional distillation and the percentages were 69.3%, 79.9%, 85.4%, and 99.5%. The different OPA fractions are termed OPA-69.3, OPA-79.9, OPA-85.4, and dillapiol-99.5 hereafter, for OPA 1, 2, 3 and 4, respectively, considering the percentage of dillapiol in the obtained fractions.

### 3.2 Phytotoxicity of OPA against *C. sinensis* and definition of working concentrations

The proportion of plants with the same degree of toxicity observed in the first assessment (1 DAA) remained constant during the entire experimental period.

Treatment of *C. sinensis* plants with OPA-69.3 at dilutions of 0.75% and 1.0%, OPA-79.9 at 0.75% dilution, and OPA-85.4 at 0.5%, 0.75%, and 1.0% dilutions caused no phytotoxic effect in 100% of the plants. OPA-69.3 at 0.5% was nontoxic to 88.88% of the plants and OPA-79.9 at 0.5% and 1.0% was nontoxic for 66.6% and 33.33% of the plants, respectively.

OPA-69.3 at 0.5% caused mild toxicity to 11.11% of the plants and OPA-79.9 at 0.5% and 1.0% caused mild toxicity to 22.22% and 33.33% of the plants, respectively.

OPA-79.9 at 0.5% and 1.0% was moderately toxic to 11.18 and 33.33% of the plants, respectively, while that of dillapiol-99.5 at 0.5% and 1.0% caused moderate toxicity to 33.66% of the plants, respectively. Dillapiol-99.5 at 0.75% caused moderate toxicity to 66.66% of the plants.

Only dillapiol-99.5 was highly toxic to plants. The 0.5 and 1.0% dilutions were highly toxic to 66.6% of the plants, while the 0.75% dilution caused high toxicity to 33.33% of the plants. The two control treatments, with or without adjuvant, were nontoxic to 100% of the plants of *C. sinensis*. Because dillapiol-99.5 presented moderate toxicity to more than 35% of the plants and was the only treatment that caused high toxicity, it was excluded from the efficacy tests on *D. citri*.

### 3.3 Assessment of the efficacy of the OPA against nymphs and adults of *D. citri* by topical contact

The nymphs of *D. citri* displayed high sensitivity to all treatments containing OPA. The average mortality obtained by the treatments varied between 90.00% and 98.57% on the first day of assessment (1 DAA), between 91.42 and 100% at 3 DAA, and between and 97.14% and 100.0% in the final assessment (7 DAA) (Table 3). The high mortality (90.00–98.57%) observed at 1 DAA in all treatments indicates a knockdown effect of the OPA for third-instar nymphs of *D. citri*. The average mortality did not significantly differ among the treatments and compared to the positive control imidacloprid. Only the lowest concentration evaluated, OPA-69.3 at 0.5%, showed a significantly higher mortality at 7 DAA compared to 1 and 3 DAA. However, the average mortality obtained by the treatments significantly differed from the control treatment at all time points (1 DAA: $F = 44.10$; $df = 11$, $P < 0.0001$; 3 DAA: $F = 35.80$; $df = 11$, $P < 0.0001$,

and 7 DAA: F = 35.87; df = 11, $P < 0.0001$). The mortality induced by the control with adjuvant was significantly higher than caused by the control containing water alone at all time points (Table 3).

In adults, OPA-79.9 at 0.75% and 1.0% dilutions and 1.0% OPA-81.6 induced the highest mortality (> 72.5%) at 1 DAA, indicating a knockdown effect. The effects did not differ from those obtained by imidacloprid, but were significantly different from those of the controls (F = 18.21; df = 11; $P < 0.0001$). In the adult insects, the effect of the control containing water alone did not differ significantly from that of the control containing adjuvant. Treatment with OPA-69.3 at a concentration of 1.0% showed intermediate efficacy; the mortality was significantly higher than that induced by the control (46.25%), but lower than that induced by imidacloprid and the higher OPA concentrations (F = 18.21; df = 11; $P < 0.0001$). The other treatments did not differ from the controls (Table 4). At 3 DAA, OPA-79.9 at 0.75% and 1.0% dilutions and the 1.0% OPA-85.4 dilution sustained the efficacy observed in the first assessment, displaying higher values of mortality (78.75–97.50%). Again, the effects did not differ from those obtained by imidacloprid, but were significantly different from those of the controls (F = 18.19; df = 11; $P < 0.0001$). The efficacies of OPA-69.3 at 1.0% and OPA-85.4 at 0.75% were intermediate, since they caused significantly higher mortality (55% and 57.50%, respectively) compared to the controls (2.5%), but were less effective than imidacloprid (98.75%) (Table 4).

At 7 DAA, OPA-69.3 at 1.0%, OPA-79.9 at 0.75% and 1.0%, and OPA-85.4 at 0.75% and 1.0% did not significantly differ from imidacloprid, with the average mortality ranging between 62.5–96.26%; however, they significantly differed from the controls (F = 17.25; df = 11; $P < 0.0001$) (Table 4). The other treatments did not significantly differ from the controls (Table 4).

Comparison of the mortality caused at different time points by each treatment revealed that the mortality did not significantly differ between the time points for 1% OPA-79.9 ($F = 1.75$; df = 2; $P = 0.1767$) and 1% OPA-85.4 ($F = 0.17$; df = 2; $P = 0.8443$). These treatments induced high mortality in *D. citri* adults (> 88.75%) at 1 DAA, not significantly differing from the other time points of assessment. In contrast, the effects of OPA-69.3 at 1.0% and OPA-79.9 at 0.75% significantly increased over the experimental period as indicated by the increasing mortality ($F = 15.67$; df = 2; $P < 0.0001$ and $F = 5.14$; df = 2; $P = 0.0068$), respectively, indicating occurrence of a lethal action after a longer period, which may be considered an intermediate effect in comparison to more effective treatments. The 0.5% OPA-79.9 and OPA-85.4 treatments also displayed a significant increase in efficacy, as indicated by the significantly increased mortality, during the experimental period ($F = 5.25$; df = 2; $P = 0.0061$ and $F = 8.30$; df = 2; $P = 0.004$); however, their efficacy remained low in the final assessment (mortality < 50%) (Table 4).

Next, we tested the interactions between the concentration of dillapiol in the OPA and the dilutions used in the treatments. An increase in the dillapiol content was reflected in a higher mortality of adults for the 0.75% ($F =1 1.05$, df = 2; $P < 0.0001$) and 1% ($F = 9.88$; df = 2; $P = 0.0001$) dilutions. However, for the 0.5% dilution, no significant difference in mortality in *D. citri* adults was observed ($F = 2.81$; df = 2; $P = 0.0659$) with increasing dillapiol content in the diluted oil extracts (Table 5). When comparing the effects of different concentrations of dillapiol for each oil extract dilution, we observed that OPA-69.3 caused significantly higher mortality at dilutions of 0.5% and 1.0%, but no significant difference was noted between 0.5% and 0.75% ($F = 3.09$; df = 2; $P = 0.0509$). OPA-79.9 caused significantly higher mortality at dilutions of 0.75% and 1.0% than at 0.5% dilution ($F = 17.54$; df = 2; $P < 0.0001$). Finally, OPA-85.4 caused

significantly increased mortality with each decrease in dilution (F = 29.22; df = 2; $P < 0.0001$) (Table 5).

### 3.4 Assessment of the residual efficacy of the OPA on *D. citri* adults

The residual efficacy of all the treatments against adult *D. citri*, regardless of the concentration of dillapiol in the OPA and the dilution used, was significantly lower than that of imidacloprid at 1, 3, and 7 DAA (F = 60.20; df = 5; $P < 0.0001$, F = 71.40; df = 5; $P < 0.0001$, and F = 64.56; df = 5; $P < 0.0001$, respectively). The treatments with OPA showed a significant increase in mortality over the assessment period (F = 9.34; df = 2, $P = 0.0002$, F = 9.74; df = 2, $P = 0.0002$, and F= 9.14; df = 2, $P = 0.0003$ for 1, 3, and 7 DAA, respectively); however, the efficacy remained low, even at 7 DAA (average mortality ≤ 30%) (Table 6).

## 4 DISCUSSION

The phytotoxicity experiment indicated that dillapiol at 99.5% was highly toxic against *C. sinensis*, independently of the dilution used. Thus, to facilitate the use of dillapiol at a high purity (99.5%), the development of new formulations would be required to mitigate this problem.

The treatments with the OPA were highly effective (mortality > 90% as soon as 1 DAA) for the control of *D. citri* nymphs in topical applications, presenting similar efficacy as imidacloprid, a widely used and effective active ingredient for control of *D. citri*. These promising results revealed its great potential for the management of this insect vector and corrobates with other studies showing that nymphs are generally sensitive to botanical insecticides.[10,33] The toxicity of the adjuvant (0.025%) to nymphs observed in this study

(approximately 50% mortality), corroborated the results of Srinivasan et al.[34] Although the authors used a 5-fold lower concentration than the one tested in our study (0.005%), the higher mortality can be attributed to the application method used by these authors, which involved immersion of nymph-infested branches in the adjuvant solution.

Regarding the efficacy of topical application against adults, we observed that various concentrations of dillapiol in OPA at different dilutions were effective for control of *D. citri* (mortality between 70–98%). OOther studies showed that neem extract at 1% dilution caused 80% mortality and reduced the number of adults on leaves up to 4-fold compared to untreated areas.[12,13] Similarly, a 1% dilution of *D. alba* extract reduced the number of *D. citri* adults on leaves by up to 4-fold compared to untreated areas.[13] Efficacies of *P. aduncum* extract against adults of the sucking insects *Aetalion* sp. and *E. herus* of 80% and 100%, respectively, have been reported after topical application at a dose of 3% and 8%, respectively.[24,33]

It is important to emphasize that in our study, we used the essential oil obtained by fractional rectification, which allows more accurate qualitative and quantitative profiling and normally has greater stability than botanical extracts. The major compounds (terpenes and terpenoids, and aromatic and aliphatic constituents) usually determine the biological propertiesof the essential.However, the activity of the major components might be modulated by other smaller molecules[35]such as apiol and myristicinthat occur in minor quantities and can exert additive or even synergistic insecticidal effects on the known insecticidal compounds such as dillapiol.[18,22,36] Because *D. citri* is an insect vector, its management requires frequent foliar insecticide spraying and the use of a reduced number of active ingredients with differentmodes ofaction, which can lead to selection of psyllid population resistant to the insecticides commonly

used for their control.[37,38] Therefore, searching for new active ingredients to be used in rotation to control this insect vector is a constant need.

The OPA is mainly composed of dillapiol,[14,19,20] which has a potential inhibitory activity against the detoxifying enzymes responsible for the elimination of plant metabolites potentially toxic to insects.[21,22] Previous studies have demonstrated that elevated levels of esterases, glutathione *S*-transferase, and cytochrome P450 enzymes are responsible for the detoxification of insecticides in *D. citri* nymphs and adults, and these enzymes are associated with lower susceptibility of this insect to insecticides frequently used for their control.[39–41]

Studies have demonstrated that dillapiol acts as a potent synergist of synthetic and botanical insecticides in agricultural pest control.[42,43] Liu et al.[43] observed that dillapiol in combination with pyrethrum extract purified from *Chrysanthemum cinerariifolium* was 9.1-fold more effective for the control of *Leptinotarsa decemlineata* (Say) larvae resistant to insecticides including pyrethrum. . Mukherjee et al.[44] showed that acyl derivatives of dihydrodillapiol have a synergistic activity towards pyrethrum against *Tribolium castaneum* (Herbst.) with a synergism factor ($LC_{50}$ for pyrethrum/$LC_{50}$ pyrethrum plus synergist) of 2.3–4.0. Shankarganesh et al.[45] reported that dihydrodillapiol combined with pyrethroids caused significant reduction in resistance of *Spodoptera litura* (F.) that is currently resistant to cypermethrin, lambda cyhalothrin, and profenophos. Tomar et al.[46] observed that a mixture of pyrethrum and dillapiol synthesized by chemical transformation (1:5) showed a synergism factor varying from 2.0 to 5.0 folds when compared to pyrethrum alone against *T. castaneum.* Thus, essential oils rich in dillapiol might help decrease resistance in *D. citri* populations, because this active ingredient can potentially inhibit the activity of detoxifying enzymes in insects. However, further studies are required to prove this hypothesis. The present study clearly demonstrated the high efficacy of the

OPA in the control of *D. citri*. As the mode of action is unlike that of insecticides commonly used in citriculture, it could be used in rotation for effective management of *D. citri.* The results of this study will contribute to the future adoption of dillapiol-rich oils as a control strategy of *D. citri*.

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**Table 1.** Treatments, dilutions, and percentage of active ingredient for each concentration of mix sprayed on sweet orange (*C. sinensis*) plants.

| Treatments | Dilution v $v^{-1}$ | % a.i. $L^{-1}$ |
|---|---|---|
| 1. Control (Water) [1,2,3] | ** | ** |
| 2. Control (Water + Adjuvant) [1,2,3] | ** | ** |
| 3. OPA 69.3% dillapiol [1,2] | 0.5 | 0.3260 |
| 4. OPA 69.3% dillapiol [1,2] | 0.75 | 0.4890 |
| 5. OPA 69.3% dillapiol [1,2,3] | 1.00 | 0.6520 |
| 6. OPA 79.9% dillapiol [1,2] | 0.5 | 0.383 |
| 7. OPA 79.9% dillapiol [1,2] | 0.75 | 0.5745 |
| 8. OPA 79.9% dillapiol [1,2,3] | 1.00 | 0.766 |
| 9. OPA 85.4% dillapiol [1,2] | 0.5 | 0.408 |
| 10. OPA 85.4% dillapiol [1,2] | 0.75 | 0.6120 |
| 11. OPA 85.4% dillapiol [1,2,3] | 1.0 | 0.8160 |
| 12. Dillapiol 99.5% [1] | 0.5 | 0.5 |
| 13. Dillapiol 99.5% [1] | 0.75 | 0.75 |
| 14. Dillapiol 99.5% [1] | 1.00 | 1.00 |
| 15. Imidacloprid [2,3] | 0.004 | 20.0 |

[1] Phytotoxicity against *C. sinensis.*

[2] Topical application on nymphs and adults of *D. citri.*

[3] Residual application on adults of *D. citri.*

**Table 2.** The composition of the essential oils of *Piper aduncum*.

| Compound | RT[a] (min.) | RI[b] | Relative area (% ± SE)[c] | | | |
|---|---|---|---|---|---|---|
| | | | OPA 1 | OPA 2 | OPA 3 | OPA 4 |
| *α*-pinene | 3.123 | 917 | 1.08 ± 0.07 | 0.55 ± 0.04 | tr | – |
| camphene | 3.385 | 931 | tr[d] | tr | – | – |
| *β*-pinene | 3.957 | 962 | 1.92 ± 0.09 | 1.01 ± 0.06 | tr | – |
| myrcene | 4.422 | 986 | tr | tr | – | – |
| *α*-phellandrene | 4.634 | 997 | 0.90 ± 0.04 | 0.30 ± 0.03 | – | – |
| *p*-cymene | 5.149 | 1018 | tr | tr | – | – |
| limonene | 5.241 | 1021 | 0.48 ± 0.02 | 0.17 ± 0.00 | – | – |
| (*Z*)-β-ocimene | 5.616 | 1035 | 0.79 ± 0.02 | 0.21 ± 0.01 | – | – |
| (*E*)-β-ocimene | 5.878 | 1045 | 1.87 ± 0.05 | 0.46 ± 0.03 | – | – |
| terpinolene | 6.886 | 1083 | tr | tr | – | – |
| *α*-cubebene | 14.907 | 1342 | 0.10 ± 0.00 | tr | tr | – |
| *α*-longipinene | 15.193 | 1351 | tr | tr | tr | – |
| cyclosativene | 15.297 | 1354 | 0.17 ± 0.01 | 0.12 ± 0.00 | tr | – |
| *α*-copaene | 15.634 | 1365 | 0.98 ± 0.01 | 0.73 ± 0.01 | 0.63 ± 0.01 | – |
| *β*-cubebene | 16.127 | 1381 | tr | tr | – | – |
| *β*-elemene | 16.203 | 1383 | 0.27 ± 0.00 | tr | 0.10 ± 0.01 | – |
| *α*-gurjunene | 16.620 | 1397 | 0.19 ± 0.00 | tr | tr | – |
| (*E*)-caryophyllene | 16.879 | 1405 | 10.44 ± 0.12 | 7.14 ± 0.08 | 4.85 ± 0.06 | – |
| *α*-santalene | 17.058 | 1411 | 0.19 ± 0.01 | 0.14 ± 0.00 | 0.10 ± 0.01 | – |
| *β*-copaene | 17.196 | 1416 | tr | tr | tr | – |
| aromadendrene | 17.441 | 1424 | 0.24 ± 0.00 | 0.10 ± 0.00 | tr | – |
| *α*-humulene | 17.872 | 1439 | 1.40 ± 0.02 | 0.93 ± 0.04 | 0.68 ± 0.00 | – |
| *allo*-aromadendrene | 18.084 | 1446 | 0.24 ± 0.01 | 0.17 ± 0.00 | 0.13 ± 0.01 | – |
| dauca-5,8-diene | 18.583 | 1463 | 0.10 ± 0.01 | tr | tr | – |
| germacrene D | 18.720 | 1467 | 0.99 ± 0.01 | 0.80 ± 0.02 | 0.21 ± 0.01 | – |
| bicyclogermacrene | 19.187 | 1483 | 1.31 ± 0.02 | 0.86 ± 0.01 | 0.28 ± 0.01 | – |
| *α*-muurolene | 19.412 | 1490 | 0.34 ± 0.01 | 0.24 ± 0.01 | 0.15 ± 0.01 | – |
| *β*-himachalene | 19.595 | 1496 | 0.72 ± 0.01 | 0.34 ± 0.01 | 0.13 ± 0.02 | – |
| *n*-pentadecane | 19.718 | 1501 | 0.96 ± 0.03 | 1.34 ± 0.01 | 2.01 ± 0.10 | – |
| *δ*-amorphene | 19.770 | 1496 | 0.24 ± 0.01 | 0.19 ± 0.01 | 0.46 ± 0.05 | – |
| *β*-curcumene | 19.870 | 1506 | 0.26 ± 0.00 | 0.17 ± 0.00 | 0.18 ± 0.00 | – |
| myristicin | 20.097 | 1514 | 2.68 ± 0.01 | 2.13 ± 0.02 | 2.06 ± 0.04 | 0.17 ± 0.02 |
| *α*-calacorene | 20.534 | 1529 | 0.15 ± 0.00 | 0.12 ± 0.02 | 0.30 ± 0.01 | – |
| germacrene B | 20.835 | 1540 | 0.10 ± 0.00 | tr | tr | – |
| *z*-isoelemicin | 21.333 | 1557 | 0.15 ± 0.00 | 0.13 ± 0.01 | 0.12 ± 0.01 | tr |
| spathulenol | 21.459 | 1562 | 0.12 ± 0.01 | – | – | – |
| caryophyllene oxide | 21.549 | 1565 | 0.29 ± 0.01 | 0.61 ± 0.03 | 0.77 ± 0.04 | tr |
| globulol | 21.822 | 1575 | 0.47 ± 0.01 | 0.45 ± 0.01 | 0.72 ± 0.04 | tr |
| dillapiol | 23.128 | 1622 | 69.3 ± 0.40 | 79.9 ± 0.10 | 85.4 ± 0.21 | 99.5 ± 0.03 |
| apiol | 24.570 | 1675 | 0.15 ± 0.00 | 0.18 ± 0.02 | 0.23 ± 0.01 | 0.15 ± 0.00 |

[a]RT = retention time on the Rxi-5MS (10 m X 0.10 mm ID X 0.10 µm) column;

[b]RI = retention index as determine on an Rxi-5MS column using the homologous series of *n*-hydrocarbons ($C_8$-$C_{20}$);

[c]Expressed as area % mean number ± SE from GC-MS data;

[d]tr = Traces (<0.1 %).

**Table 3.** Average mortality (± SEM) of third instar nymphs of *Diaphorina citri* in topical application of different concentrations of dillapiol and dilutions of the essential oil of *Piper aduncum.*

| Treatments | Dilution v v$^{-1}$ | n | Mortality (%) | | |
|---|---|---|---|---|---|
| | | | 1 DAA | 3 DAA | 7 DAA |
| **Control (water)** | ** | 7 | 1.42 ± 1.42Cb | 10.00 ± 5.34Ca | 10.00 ± 5.34Ca |
| **Control (adj)** | 0.025 | 7 | 35.71 ± 10.43Bb | 45.71 ± 10.87Ba | 47.14 ± 10.62Ba |
| **OPA 69.3% dillapiol** | 0.5 | 7 | 90.00 ± 5.34Ab | 91.42 ± 5.53Ab | 97.14 ± 1.84Aa |
| **OPA 69.3% dillapiol** | 0.75 | 7 | 100.00 ± 0.00Aa | 100.00 ± 0.00Aa | 100.00 ± 0.00Aa |
| **OPA 69.3% dillapiol** | 1.00 | 7 | 95.71 ± 2.97Aa | 97.14 ± 1.84Aa | 98.57 ± 0.00Aa |
| **OPA 79.9% dillapiol** | 0.5 | 7 | 94.28 ± 5.71Aa | 97.14 ± 2.85Aa | 97.14 ± 2.85Aa |
| **OPA 79.9% dillapiol** | 0.75 | 7 | 98.57 ± 1.42Aa | 100.00 ± 0.00Aa | 100.00 ± 0.00Aa |
| **OPA 79.9% dillapiol** | 1.00 | 7 | 97.14 ± 2.85Aa | 97.14 ± 2.85Aa | 97.14 ± 2.85Aa |
| **OPA 85.4% dillapiol** | 0.5 | 7 | 97.14 ± 2.85Aa | 97.14 ± 2.85Aa | 97.14 ± 2.85Aa |
| **OPA 85.4% dillapiol** | 0.75 | 7 | 91.42 ± 8.57Aa | 91.42 ± 8.57Aa | 91.42 ± 8.57Aa |
| **OPA 85.4% dillapiol** | 1.00 | 7 | 98.57 ± 1.42Aa | 100.00 ± 0.00Aa | 100.00 ± 0.00Aa |
| **Imidacloprid** | 0.004 | 7 | 100.00 ± 0.00Aa | 100.00 ± 0.00Aa | 100.00 ± 1.42Aa |

Means followed by same capital letter in the column and lowercase in the row do not differ as indicated by Tukey's test ($P \leq 0.05$).

**Table 4.** Average mortality (± SEM) of adults of *Diaphorina citri* in topical application of different concentrations of dillapiol and dilutions of the essential oil of *Piper aduncum.*

| Treatments | Concentration v v$^{-1}$ | n | Mortality (%) | | |
|---|---|---|---|---|---|
| | | | 1 DAA | 3 DAA | 7 DAA |
| **Control (water)** | ** | 8 | 1.25 ± 1.25 Da | 2.50 ± 1.63 Ea | 6.25 ± 2.63 Da |
| **Control (adj)** | 0.025 | 8 | 5.00 ± 2.67 Da | 11.25 ± 4.79 Ea | 13.75 ± 4.60 Da |
| **OPA 69.3% dillapiol** | 0.5 | 8 | 33.75 ± 12.94 BCDa | 36.25 ± 13.22 DEa | 43.75 ± 12.94 BCDa |
| **OPA 69.3% dillapiol** | 0.75 | 8 | 16.25 ± 7.54 CDa | 17.50 ± 7.73 DEa | 21.25 ± 7.42 Da |
| **OPA 69.3% dillapiol** | 1.00 | 8 | 46.25 ± 14.99 BCb | 55.00 ± 14.26 CDb | 70.00 ± 10.00 ABa |
| **OPA 79.9% dillapiol** | 0.5 | 8 | 20.00 ± 9.25 CDb | 25.00 ± 9.06 DEab | 33.75 ± 9.43 BCDa |
| **OPA 79.9% dillapiol** | 0.75 | 8 | 72.50 ± 11.76 ABb | 78.75 ± 10.92 ABCab | 86.25 ± 9.80 Aa |
| **OPA 79.9% dillapiol** | 1.00 | 8 | 88.75 ± 6.39 Aa | 95.00 ± 3.77 ABCa | 96.25 ± 2.63 Aa |
| **OPA 85.4% dillapiol** | 0.5 | 8 | 5.00 ± 3.77 Db | 13.75 ± 7.05 Eab | 22.50 ± 8.60 CDa |
| **OPA 85.4% dillapiol** | 0.75 | 8 | 36.25 ± 10.84 BCDb | 57.50 ± 13.59 BCDa | 62.50 ± 13.59 ABCa |
| **OPA 85.4% dillapiol** | 1.00 | 8 | 96.25 ± 3.75 Aa | 97.50 ± 2.50 ABa | 98.75 ± 1.25 Aa |
| **Imidacloprid** | 0.004 | 8 | 95.00 ± 3.77 Aa | 98.75 ± 1.25 Aa | 100.00 ± 0.00 Aa |

Means followed by same capital letter in the column and lowercase in the row do not differ as indicated by Tukey's test ($P \leq 0.05$).

**Table 5.** Influence of the concentration of dillapiol and dilution of the essential oil of *Piper aduncum* on the percentage of mortality (± SEM) of *Diaphorina citri* adults in topical application.

| Dillapiol (%) | N | Essential oil (v $v^{-1}$) | | |
|---|---|---|---|---|
| | | **0.5** | **0.75** | **1.00** |
| **69.3%** | 8 | 43.75 ± 12.94 Aab | 21.25 ± 7.42 Bb | 70.00 ± 10.00 Ba |
| **79.9%** | 8 | 33.75 ± 9.43 Ab | 86.25 ± 9.80 Aa | 96.25 ± 2.63 Aa |
| **85.4%** | 8 | 22.50 ± 8.60 Ac | 62.50 ± 13.59 Ab | 98.75 ± 1.25 Aa |

Means followed by same capital letter in the column and lowercase in the row do not differ as indicated by Tukey's test ($P \leq 0.05$).

Values in the table correspond to final time point (7 DAA).

**Table 6.** Average mortality (± SEM) of *Diaphorina citri* adults after residual application of different concentrations of dillapiol and dilutions of the essential oil of *Piper aduncum.*

| Treatments | Concentration v v$^{-1}$ | n | Mortality (%) | | |
|---|---|---|---|---|---|
| | | | 1 DAA | 3 DAA | 7 DAA |
| **Control (water)** | ** | 8 | 10.00 ± 4.62 Ba | 14.28 ± 4.16 BCa | 17.14 ±4.51 BCa |
| **Control (adj)** | 0.025 | 8 | 0.00 ± 0.00 Ba | 2.50 ± 1.63 Ca | 5.00 ±3.27 Ca |
| **Dillapiol 69.3%** | 1.00 | 8 | 5.00 ± 1.88 Bb | 10.00 ± 4.62 BCb | 18.75 ±5.49 BCa |
| **Dillapiol 79.9%** | 1.00 | 8 | 3.75 ± 2.63 Bb | 7.50 ± 4.11 BCb | 17.50 ±4.53 BCa |
| **Dillapiol 85.4%** | 1.00 | 8 | 16.25 ± 5.95 Bb | 22.50 ± 5.59 Bab | 30.00 ±8.01 Ba |
| **Imidacloprid** | 0.004 | 8 | 87.50 ± 5.26 Ab | 98.75 ± 1.25 Aa | 100.00 ±0.00 Aa |

Means followed by same capital letter in the column and lowercase in the row do not differ by as indicated by Tukey's test ($P \leq 0.05$).