Bioassay of Plant Extracts Against Aleurodicus dispersus (Hemiptera: Aleyrodidae)

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Bioassay of plant extracts against *Aleurodicus dispersus* (Hemiptera: Aleyrodidae)

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**Abstract**

The spiraling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), is a destructive invasive pest in many parts of the world. Topical spray and dry film contact assays were conducted to measure the toxicity of 8 plant extracts and their mixtures traditionally used as insecticides in South Asian countries such as Bangladesh, India, and Nepal. The highest mortality (100%) of adults was recorded for neem (*Azadirachta indica* A. Juss.; Meliaceae) (ethanol) extract (500 mg/L) at 6 h after topical spray. This was followed by 5-leafed chaste tree (*Vitex negundo* L.; Lamiaceae) (ethanol), sweet sop (*Annona squamosa* L.; Annonaceae) (acetone), water pepper (*Polygonum hydropiper* L.; Polygonaceae) (acetone), banyan (*Ficus benghalensis* L.; Moraceae) (ethanol), banyan (acetone), and crown flower (*Calotropis gigantea* L. W. T. Aiton; Apocynaceae) (ethanol) at 18 h after the treatment. Bioassay results indicate that neem (ethanol) extract mixed with crown flower (acetone), oleander (*Nerium indicum* Mill.; Apocynaceae) (acetone), or sweet sop (ethanol) (in the ratio of 1:1, 1:2, and 1:3 for each plant extract) showed synergism. Neem (ethanol) extract also showed the highest mean repellency rate (93%). In conclusion, neem, 5-leafed chaste tree, sweet sor, water pepper, banyan, and crown flower extracts showed good potential to control *A. dispersus*, and the mixtures of these plant extracts showed synergistic activity against *A. dispersus*.

**Key Words:** direct spray; residual film; mixed extract; antifeedant; mutual effect

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**Resumen**

La mosca blanca en espiral, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), es una plaga invasiva destructiva en muchas partes del mundo. Se realizaron ensayos de contacto tópico y de película seca con una planta de seco para medir la toxicidad de 8 extractos de plantas y sus mezclas tradicionalmente usadas como insecticidas en países del sur de Asia tales como Bangladesh, India y Nepal. La mayor mortalidad (100%) de los adultos se registró para el extracto de neem (*Azadirachta indica* A. Juss; Meliaceae) (etanol) (500 mg/L) a las 6 horas después de las aspersiones tópicas. A esto le siguió el árbol casto de 5 hojas (*Vitex negundo* L.; Lamiaceae) (etanol), anón dulce (*Annona squamosa* L.; Annonaceae) (acetona), pimienta de agua (*Polygonum hydropiper* L.; Polygonaceae) (etanol), higuera (*Ficus benghalensis* L.; Moraceae) (acetona) y flor de corona (*Calotropis gigantea* L. W. T. Aiton; Apocynaceae) (etanol) en 500 mg/L a las 12 horas después de las aspersiones. Para el método de la película seca, la mayor mortalidad (100%) de los adultos también se registró para el extracto de neem (etanol) (500 mg/L) a las 18 h después del tratamiento. Los resultados del bioensayo indican que el extracto de neem (etanol) mezclado con flor de corona (acetona), adelfa (*Nerium indicum* L.; Apocynaceae) (acetona), o anón dulce (etanol) (en la proporción de 1:1, 1:2 y 1:3 para cada extracto de planta) mostró sinergismo. El extracto de neem (etanol) también mostró la mayor tasa de repelencia media (93%). En conclusión, el neem, el árbol casto de 5 hojas, el anón dulce, la pimienta de agua, el banyan (acetona), el banyan (etanol) y los extractos de flor corona mostraron un buen potencial para controlar *A. dispersus* y las mezclas de estos extractos vegetales mostraron actividad sinérgica contra *A. dispersus*.

**Palabras Clave:** aspersiones directas; película residual; extracto mixto; anti-alimentación; efecto mutuo

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Severe infestations of this pest are a serious threat to guava production directly damaging the plant by removing sap from leaves, which reduces growth, weakens plants, and reduces crop yield (Nasruddin & Stocks 2014). Whiteflies also reduce the photosynthetic area of the leaves and lower the market value of fruit due to cosmetic damage from sooty mold (Bryne et al. 1990; Liu et al. 2007). Sooty mold may increase thermal absorption and raise leaf temperature, reducing leaf efficiency and causing premature tissue death (Bryne et al. 1990). Serious infestations of *A. dispersus* have been found on important ornamental plants, including poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch; Euphorbiaceae) and madhobilotra (*Hiptage benghalensis* L. Kurz; Malpighiaceae) in Bangladesh (Alim et al. 2014).
Materials and Methods

INSECT REARING

The adults of *A. dispersus* used in assays were obtained from guava trees where they were managed without use of insecticides in guava orchards. *Aleurodius dispersus* used in our bioassays were adults at least 24 h old collected randomly from a population of several hundred individuals maintained on guava branches (temperature 26.2 °C and RH 84%). To produce insects for tests, infested guava branches were combined with whitefly-free guava twigs for 24 h. The new twigs infested with whitefly eggs were then covered with mosquito netting (22 cm L × 15 cm D) and held for whitefly development. Branches were observed daily for whitefly pupation, and new adults were collected as they emerged and used in subsequent bioassays.

PLANT MATERIALS USED FOR BIOASSAYS

Eight plant species from Bangladesh and India that are known to have insecticidal activity (Rajashekar et al. 2012) were identified from their natural habitats from Dinajpur District, Bangladesh, and their leaves were used in our bioassays (Table 1). The identity of each plant species was verified and confirmed by plant morphology (Karim & Kabir 1995).

Table 1. Toxicity of extracts from 8 plant species against *Aleurodius dispersus* adults by topical spray.

| Common name | Scientific name (family) | Solvent | Dose (mg/L) | 6    | 12   | 18   |
|-------------|--------------------------|---------|-------------|------|------|------|
| Neem/Margosa | *Azadirachta indica* (Meliaceae) | Ethanol | 500         | —   | —   | —   |
|             |                          |         | 400         | 77.8 (0.23) a–h | 91.1 (2.88) ab | 93.3 (3.36) ab |
|             |                          |         | 500         | 51.1 (0.56) e–n | 73.8 (1.85) abc | 100 a |
|             |                          |         | 400         | 75.6 (0.09) i–i | 86.7 (2.71) ab | 97.7 (3.27) ab |
| Five-leaved chaste tree | *Vitex negundo* (Lamiaceae) | Ethanol | 500         | 93.3 (0.15) a–c | 100 a | —   |
|             |                          |         | 400         | 55.6 (0.23) d–m | 80.0 (2.07) abc | 93.3 (2.84) ab |
|             |                          |         | 500         | 57.6 (0.31) c–m | 91.1 (2.26) ab | 97.8 (3.40) ab |
|             |                          |         | 400         | 40.0 (0.30) i–n | 84.4 (1.34) ab | 95.6 (3.18) ab |
| Sweet sop   | *Annona squamosa* (Annonaceae) | Ethanol | 500         | 46.7 (0.15) g–n | 89.9 (2.17) ab | 93.3 (3.36) ab |
|             |                          |         | 400         | 48.9 (0.23) f–n | 64.4 (1.85) a–d | 73.3 (2.32) ab |
|             |                          |         | 500         | 95.6 (0.17) ab | 100 a | —   |
|             |                          |         | 400         | 86.7 (0.15) a–e | 97.8 (3.27) a | 100 a |
| Oleander    | *Nerium indicum* (Apocynaceae) | Ethanol | 500         | 91.1 (0.23) a–d | 95.6 (3.45) a | 100 a |
|             |                          |         | 400         | 75.6 (0.23) a–i | 91.1 (2.88) ab | 95.6 (3.44) ab |
|             |                          |         | 500         | 57.8 (0.34) c–m | 86.7 (2.07) ab | 97.8 (3.15) ab |
|             |                          |         | 400         | 53.3 (0.15) e–m | 75.6 (2.15) abc | 84.4 (2.75) ab |
| China box   | *Buxus sinica* (Buxaceae) | Ethanol | 500         | 91.1 (0.34) a–d | 95.5 (3.45) a | 100 a |
|             |                          |         | 400         | 80.0 (0.15) a–g | 88.9 (3.06) ab | 93.3 (3.36) ab |
|             |                          |         | 500         | 86.7 (0.39) a–e | 95.6 (3.57) a | 100 a |
|             |                          |         | 400         | 71.1 (0.23) a–j | 84.4 (2.75) ab | 93.3 (3.23) ab |
| Water pepper| *Polygonum hydropiper* (Polygonaceae) | Ethanol | 500         | 71.1 (0.17) a–j | 91.1 (2.63) ab | 100 a |
|             |                          |         | 400         | 51.1 (0.23) e–n | 71.1 (1.85) a–d | 82.2 (2.67) ab |
|             |                          |         | 500         | 95.6 (0.09) ab | 100 a | —   |
| Banyan      | *Ficus benghalensis* (Moraceae) | Ethanol | 500         | 77.8 (0.23) a–h | 89.9 (2.80) ab | 93.3 (3.36) ab |
|             |                          |         | 400         | 75.6 (0.09) a–i | 86.7 (2.84) ab | 100 a |
|             |                          |         | 500         | 95.6 (0.17) ab | 100 a | —   |
| Crown flower| *Calotropis gigantea* (Apocynaceae) | Ethanol | 500         | 77.8 (0.23) a–h | 86.7 (2.97) ab | 95.6 (3.61) ab |
|             |                          |         | 400         | 48.9 (0.23) f–n | 64.4 (1.85) a–d | 73.3 (2.32) ab |
|             |                          |         | 500         | 95.6 (0.17) ab | 100 a | —   |
|             |                          |         | 400         | 86.7 (0.15) a–e | 97.8 (3.27) a | 100 a |

*Means (± SE) in a column followed by different letters indicate significant differences from the Tukey test (α = 0.05).*
PREPARATION OF PLANT EXTRACTS

Fresh leaves for extraction were collected from mature plants (after the fruiting stage), cut into pieces, and ground in a mortar with a pestle. Ten grams of ground leaves of a particular species were mixed with 100 mL distilled water at room temperature (27.7 ± 0.2 °C; 75 ± 10% RH), and the mixture was passed through filter paper (Whatman® No. 1, Sigma-Aldrich, Inc. Germany). Twenty-five mL of this filtrate were mixed with 100 mL of 1 of 2 solvents (ethanol or acetone) (Abe & Matsuda 2000). Each mixture was shaken for 30 min and left to separate in a funnel (V × L: 250 mL × 345 mm) with a conical flask (200 mL). After collection of the relevant fraction from the separating funnel, the extract was transferred to a flask and condensed by evaporating the solvent in a rotary evaporator (D 79219, Ika Werke Gmbh and Co., Staufen, Germany) at 78 °C or 56 °C, for ethanol and acetone extracts, respectively. The crude extract was then transferred quantitatively to a clean, weighed vial and kept in a refrigerator (3.0 ± 0.2 °C) until used for assays.

BIOASSAYS WITH A. DISPERSUS ADULTS

Topical Method

Test solutions from each of the 32 plant extracts were prepared in 10 mL batches (400 and 500 mg/L with 50% acetone in water). To prepare a test arena, a guava leaf petiole was inserted into moistened cotton and placed in a Petri dish (15 cm D × 3 cm H) with the ventral surface facing upward. Adults of A. dispersus (24 h old) from the rearing colony were collected in a group and placed on ice to allow counting. Batches of 20 cold-immobilized adults were placed on test leaves in Petri dishes with a fine brush and allowed to warm and regain mobility. When 15 insects had recovered from cold-immobilisation and resumed activity, 1 mL of the 400 mg/L test solution was sprayed onto the leaf with a spray tower (Potter, Burkard Company, Oxbridge, United Kingdom). After air-drying, each Petri dish was closed with a lid and sealed with Parafilm®. Control Petri dishes were treated with an acetone (50% in water) solution. Each test was replicated 3 times. Treated adult whiteflies were examined for mortality at 6 h intervals after the treatment for 3 d. Adults that did not respond to probing with a small brush were considered dead. These procedures were conducted for the 8 acetone extracts and for the 8 ethanol extracts at concentrations of 400 and 500 mg/L.

Residual Assay Method

A guava leaf petiole was inserted into moistened cotton and placed into a Petri dish (15 cm D × 3 cm H) with the underside of the leaf facing upward. Test solutions (1 mL of the 400 or 500 mg/L solutions of either ethanol or acetone extracts) of each plant species were sprayed separately onto leaves using the spray tower as in the previous experiment, and the leaves were allowed to air-dry for 1 h. Twenty adults of A. dispersus (24 h old) were immobilized on ice as described for the previous experiment and gently placed on the treated leaves in the Petri dishes. Control Petri dishes were treated with acetone (50% in water). Each test was replicated 3 times. The treated adults were examined for mortality at 6 h intervals for 72 h after the treatment. Adults that did not show any responses when probed with a small brush were considered dead.

Tests with Mixed Extracts

The synergistic effect of mixtures of neem (ethanol) plus crown flower (acetone), neem (ethanol) plus oleander (acetone), and neem (ethanol) plus sweet sop (ethanol) extracts on A. dispersus adults was determined by the co-toxicity coefficient (CTC) method in the laboratory (Sun & Johnson 1960; Zhang et al. 2008). The concentration of 500 mg/L of 3 different mixtures at 3 ratios (1:1, 1:2, and 1:3) was applied using the spray tower to 20 A. dispersus adults, which were incubated in Petri dishes as described for the previous experiments. A control was sprayed with distilled water. Each treatment was replicated 3 times. Mortality was recorded at 6 h intervals up to 72 h after treatment.

The co-toxicity coefficient (CTC) was calculated as: LD50 of toxicant alone / LD50 of toxicant in the mixture) × 100. When CTC is 100, it indicates a probability of similar action. When CTC > 100, a synergistic action can be assumed whereas CTC < 100 indicates antagonism.

Repellency Tests

Two fully expanded guava leaves were placed in a Petri dish (15 cm D × 3 cm H) with the ventral surface facing upward. One leaf was dipped in the tested plant extract (500 mg/L) and another was dipped in acetone (50% in water). Twenty adults of A. dispersus (24 h old) were immobilized on ice and gently introduced between the 2 treated leaves in the Petri dish (5 cm D × 1 cm H). Numbers of adults resting on each leaf were recorded after 3, 6, and 24 h. Each test was replicated 3 times.

Data were expressed as percentage of repulsion (PR) as PR (%) = (Nc − Nf) × 2, where Nc = the percentage of insects present in the control. Positive (+) values indicate repellency and negative (−) values indicate attraction. Repellency class was categorized as class 0 ≤ 0.1, class I = 0.1 to 20.0, class II = 20.1 to 40.0, class III = 40.1 to 60.0, class IV = 60.1 to 80.0, and class V = 80.1 to 100.0% repellency (McGovern et al. 1977).

STATISTICAL ANALYSES

Differences in mortality among treatments were analyzed by univariate comparison testing (ANOVA) with SPSS® software version 16.0 (SPSS Inc., Chicago, IL). Post-hoc analyses were done by the Tukey test (2010). Data on lethal effects, Chi-squared values, and LT50 values were obtained from probit analysis (Robertson et al. 2007). Significant differences among the extracts in each assay were recorded when 95% confidence intervals (CI) did not overlap. LD50 values of the tested plant extracts and confidence limits were calculated for A. dispersus with log dosage–mortality probit regression equations. Co-toxicity coefficients were calculated according to Sun & Johnson (1960).

Results

TOXICITY OF PLANT EXTRACTS TO A. DISPERSUS ADULTS BY TOPICAL SPRAY

Mortality of A. dispersus adults on guava leaves sprayed with different plant extracts under laboratory conditions varied significantly by extract (Table 1). All plant extracts had an adverse effect on A. dispersus at different times after the treatment in this assay (Fig. 1A). At 6 h after the topical application of neem (500 mg/L), 100% mortality was recorded for the ethanol extract and 51% for the acetone extract. The topical application of neem (500 mg/L) caused 74% mortality at 12 h after application for the acetone extract. Five-leaved chaste tree (ethanol), sweet sop (acetone), water pepper (acetone), banyan (ethanol), banyan (acetone), and crown flower (ethanol) extracts caused 100% mortality at 12 h after topical spray (Table 1; Fig. 1A). When applied at 400 mg/L (with 50% acetone as diluent), sweet sop (acetone), banyan (ethanol), and crown flower (ethanol) extracts caused 100% mortality...
at 18 h after topical spray (Table 1; Fig. 1B). Probit analysis showed that A. dispersus was most sensitive to neem ethanol extract and least sensitive to crown flower (acetone) extract (Table 2). The mortality response to dose among extracts is summarized in Table 2.

**TOXICITY OF PLANT EXTRACTS TO A. DISPERUS ADULTS BY RESIDUAL ASSAY METHOD**

Neem (ethanol) extract at 500 mg/L (with 50% acetone as diluent) caused the highest mortality and killed 100% of A. dispersus 18 h after treatment when extracts were presented as dried residues (residual assay method) (Fig. 2A). Neem (acetone), 5-leaved chaste tree (acetone), China box (acetone), banyan (acetone), and crown flower (ethanol) extracts caused 100% mortality at 30 h after treatment with 500 mg/L (with 50% acetone as diluent). The other plant extracts were effective but caused significantly lower mortality. Based on probit analysis, neem (ethanol) extract was the most toxic to A. dispersus whereas crown flower (acetone) extract was the least toxic (Table 3; Fig. 2A). Neem (ethanol) extract caused the highest mortality of A. dispersus in the residual assay method within 24 h after treatments (Fig. 2B). Mortality responses to 500 mg/L and 400 mg/L (with 50% acetone as diluent) are summarized in Table 3.

**EFFECTS OF MIXED PLANT EXTRACTS ON A. DISPERUS ADULTS**

The effects of 3 different mixtures, neem (ethanol) plus crown flower (acetone), neem (ethanol) plus oleander (acetone), and neem (ethanol) plus sweet sop (acetone) extracts at 3 ratios (1:1, 1:2, and 1:3) on adults of A. dispersus were synergistic. Bioassay results revealed that the synergism was significant, and their co-toxicity coefficients were highest in neem (ethanol) plus crown flower (acetone) extracts (256.78) and lowest in neem (ethanol) plus oleander (acetone) extracts (102.02) at 1:3 and 1:1 ratios, respectively (Table 4).

**REPELLENCY OF PLANT EXTRACTS TO A. DISPERUS ADULTS**

The repellency of the tested plant extracts to A. dispersus adults varied (Table 5). Neem (ethanol) extract showed the highest mean repellency rate (93.3%) and belongs to repellency class V, followed by water pepper (ethanol), water pepper (acetone), and crown flower (ethanol) extracts in the same class. The 5-leaved chaste tree (acetone) and crown flower (acetone) extracts belong to repellency class IV, and the rest of the plant extracts belong to different repellency classes. Sweet sop (acetone) extract showed the lowest mean repellency rate (35.6%), belonging to repellency class II.
Table 2. Statistical comparison of LT$_{50}$ values of plant extracts against *Aleurodicus dispersus* adults tested by topical spray ($n = 45$).

| Plant extract | Solvent | Dose (mg/L) | LT$_{50}$ (h) | 95% confidence interval | Slope (± SE) | $\chi^2$ (df) | Toxicity index (TI) | LT$_{50}$ (h) | 95% confidence interval | Slope (± SE) | $\chi^2$ (df) | Toxicity index (TI) |
|---------------|---------|-------------|---------------|-------------------------|-------------|-------------|---------------------|---------------|-------------------------|-------------|-------------|---------------------|
| Neem          | Ethanol | 500         | 44.1          | —                       | —           | 44.1        | —                   | 44.1          | —                       | —           | 44.1        | —                   |
|               | Acetone | 500         | 6.3 a         | 5.0–7.3                | 3.4 (0.56)  | 4.3 (10)    | 7.0      | 3.8 a                  | 2.0–5.3       | 2.9 (0.57)              | 3.6 (10)    | 11.5        |
| F. chaste tree| Ethanol | 500         | 6.9 a         | 3.3–9.7                | 3.2 (0.40)  | 16.3 (10)   | 6.4      | 7.6 a                  | 4.0–10.5     | 2.9 (0.34)              | 14.1 (10)   | 5.8         |
|               | Acetone | 500         | 5.4 a         | 4.0–6.5                | 4.1 (0.79)  | 0.4 (10)    | 8.1      | 6.9 a                  | 5.5–8.1      | 3.9 (0.50)              | 2.6 (10)    | 6.4         |
| Sweet sop     | Ethanol | 500         | 1.1 a         | 0.1–3.8                | 2.6 (0.89)  | 1.9 (10)    | 22.5     | 3.6 a                  | 2.1–5.0      | 2.9 (0.56)              | 2.0 (10)    | 12.2        |
|               | Acetone | 500         | 5.5 a         | 4.1–6.7                | 3.9 (0.63)  | 1.3 (10)    | 8.0      | 6.2 a                  | 4.4–7.7      | 3.8 (0.54)              | 6.0 (10)    | 7.1         |
| Oleander      | Ethanol | 500         | 1.1 a         | 0.1–3.8                | 2.6 (0.89)  | 1.9 (10)    | 22.5     | 3.3 a                  | 1.4–4.8      | 2.8 (0.59)              | 3.1 (10)    | 13.5        |
|               | Acetone | 500         | 2.7 a         | 0.5–4.3                | 3.1 (0.93)  | 1.3 (10)    | 16.3     | 4.3 a                  | 2.5–5.7      | 2.8 (0.49)              | 4.0 (10)    | 10.4        |
| Water pepper  | Ethanol | 500         | 4.4 a         | 2.7–5.7                | 3.7 (0.77)  | 2.0 (10)    | 10.0     | 6.8 a                  | 4.0–9.1      | 3.2 (0.40)              | 11.0 (10)   | 6.5         |
|               | Acetone | 500         | 4.4 a         | 2.7–5.7                | 3.7 (0.77)  | 2.0 (10)    | 10.0     | 6.8 a                  | 4.0–9.1      | 3.2 (0.40)              | 11.0 (10)   | 6.5         |
| Banyan        | Ethanol | 500         | 1.1 a         | 0.1–3.8                | 2.6 (0.89)  | 1.9 (10)    | 22.5     | 3.3 a                  | 1.4–4.8      | 2.8 (0.59)              | 3.1 (10)    | 13.5        |
|               | Acetone | 500         | 4.4 a         | 2.7–5.7                | 3.7 (0.77)  | 2.0 (10)    | 10.0     | 6.8 a                  | 4.0–9.1      | 3.2 (0.40)              | 11.0 (10)   | 6.5         |
| Crown flower  | Ethanol | 500         | 7.7 a         | 6.2–9.0                | 6.2 (0.90)  | 4.9 (10)    | 5.7      | 10.0 a                 | 8.3–11.6     | 3.1 (0.32)              | 2.9 (10)    | 4.4         |
|               | Acetone | 500         | 7.7 a         | 6.2–9.0                | 6.2 (0.90)  | 4.9 (10)    | 5.7      | 10.0 a                 | 8.3–11.6     | 3.1 (0.32)              | 2.9 (10)    | 4.4         |
| Control       | Ethanol | 500         | 44.1 b        | 38.3–54.4              | 2.8 (0.41)  | 2.4 (10)    | 1.0      | 44.1 b                 | 38.3–54.4    | 2.8 (0.41)              | 2.4 (10)    | 1.0         |
|               | Acetone | 500         | 44.1 b        | 38.3–54.4              | 2.8 (0.41)  | 2.4 (10)    | 1.0      | 44.1 b                 | 38.3–54.4    | 2.8 (0.41)              | 2.4 (10)    | 1.0         |

LT$_{50}$ values in a column followed by different letters are statistically significant (Robertson et al. 2007).

*Extract killed all *A. dispersus* adults within 6 h after treatment.

*Extract killed all *A. dispersus* adults within 12 h after treatment.

*By 6 and 12 h after treatments, all insects had died, and analysis did not give LT$_{50}$ values.*
Discussion

Chemicals derived from Dalmatian pyrethrum (*Tanacetum cinerariifolium* [Trevir.] Sch. Bip.; Asteraceae), neem, and other plant species are traditionally used to control various crop pests in Asia (Saminathan & Jayaraj 2001; Liang et al. 2003; Atkinson et al. 2004). Neem products, tobacco extracts, and rosin soap have been found to be effective against *A. dispersus* in several countries such as Bangladesh, India, and Pakistan (Dubey & Sundararaj 2004; Kambrekar & Awaknavar 2004; Sinha et al. 1992; Luo et al. 2002). Zhang et al. (2008) observed that bi- and tri-weekly applications of neem extract were effective plant extracts were 5-leaved chaste tree (ethanol), sweet sop (acetone), water pepper (acetone), banyan (ethanol), banyan (acetone), and crown flower (ethanol) at 12 h after treatment at 500 mg/L. In the dry film assay, the highest mortality (100%) was also recorded from neem (ethanol) at 18 h after treatment at 500 mg/L. Dubey & Sundararaj (2004) reported that bi- and tri-weekly applications of neem effectively controlled nymphal populations of *A. dispersus*, causing 62.2% mortality even 21 d after treatment. In another study, NeemAzal® contains 10 g/L azadirachtin A.

Table 4. Comparative toxicity of mixed plant extracts to *Aleurodicus dispersus* adults.

| Plant extract                  | Ratio | LD₅₀ (mg/L) | 95% confidence interval | Regression equation | Co-toxicity coefficient |
|-------------------------------|-------|-------------|--------------------------|---------------------|-------------------------|
| Neem (ethanol)                | —     | 232.5       | 185.6–371.4              | y = −1.88 + 2.90x   | 2.5 (1)                 |
| Neem (ethanol) + crown flower (acetone) | 1:1   | 134.8       | 36.8–494.7               | y = 2.94 + 2.33x    | 2.5 (1)                 |
|                               | 1:2   | 127.2       | 27.9–581.0               | y = 0.74 + 2.00x    | 8.4 (1)                 |
|                               | 1:3   | 90.6        | 10.0–817.0               | y = 1.05 + 2.01x    | 1.2 (1)                 |
| Neem (ethanol) + oleander (acetone) | 1:1   | 227.8       | 170.0–305.2              | y = −8.57 + 5.75x   | 0.1 (1)                 |
|                               | 1:2   | 211.5       | 130.0–344.0              | y = −3.05 + 3.46x   | 0.9 (1)                 |
|                               | 1:3   | 192.0       | 101.8–362.2              | y = −2.08 + 3.10x   | 0.0 (1)                 |
| Neem (ethanol) + sweet sop (ethanol) | 1:1   | 165.8       | 67.9–404.5               | y = −0.89 + 2.65x   | 7.7 (1)                 |
|                               | 1:2   | 142.7       | 41.5–488.9               | y = 0.14 + 2.25x    | 0.2 (1)                 |
|                               | 1:3   | 132.0       | 37.7–461.7               | y = −0.68 + 2.68x   | 1.1 (1)                 |

Neem (ethanol) contains 10 g/L azadirachtin A; NeemAzal® contains 10 g/L azadirachtin A; Co-toxicity coefficient calculated using the formula: Co-toxicity coefficient = (LD₅₀ of mixed extract)/LD₅₀ of individual extract.

A. dispersus values in a column followed by different letters are statistically significant (Robertson et al. 2007).

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**Table 3.** Statistical comparison of LT₅₀ values of plant extracts against *Aleurodicus dispersus* adults tested by dry film (n = 45).

| Plant extract | Solvent | Dose (mg/L) | LT₅₀ | 95% confidence interval | χ² (df) | Toxicity index (TI) | LT₅₀ | 95% confidence interval | χ² (df) | Toxicity index (TI) |
|---------------|---------|-------------|------|-------------------------|---------|---------------------|------|-------------------------|---------|---------------------|
| Neem          | Ethanol | 500         | 4.2 a| 2.5–5.5                 | 3.5 (10)| 11.3                | 4.7 a| 3.0–6.1                 | 3.0 (10)| 10.3                |
|               | Acetone | 500         | 8.2 b| 6.1–10.2                | 13.7 (10)| 5.8                | 15.8 b| 13.4–18.1               | 4.1 (3.4)| 13.7 (10)            |
| Five-leaved chaste tree | Ethanol | 500         | 16.8 c| 11.1–21.9              | 38.2 (10)| 2.8                | 16.1 b| 12.0–19.9               | 2.7 (0.22)| 38.2 (10)            |
|               | Acetone | 500         | 12.2 c| 10.7–13.8               | 10.2 (10)| 3.9                | 23.5 b| 19.3–27.4               | 5.1 (0.38)| 10.2 (10)            |
| Sweet sop     | Ethanol | 500         | 16.9 c| 14.1–19.5               | 19.1 (10)| 2.8                | 20.3 b| 16.5–23.9               | 4.1 (0.30)| 19.1 (10)            |
|               | Acetone | 500         | 14.3 c| 11.6–16.7               | 18.0 (10)| 3.3                | 20.0 b| 15.7–24.0               | 3.2 (0.25)| 18.0 (10)            |
| Oleander      | Ethanol | 500         | 10.9 b| 8.6–13.0                | 11.5 (10)| 4.4                | 19.1 b| 14.5–23.5               | 3.2 (0.25)| 11.5 (10)            |
|               | Acetone | 500         | 17.7 c| 15.3–20.0               | 12.5 (10)| 2.7                | 19.0 b| 14.5–23.3               | 3.3 (0.25)| 12.5 (10)            |
| China box     | Ethanol | 500         | 19.1 c| 14.6–23.3               | 45.3 (10)| 2.5                | 22.8 b| 18.1–27.2               | 4.3 (0.31)| 45.3 (10)            |
|               | Acetone | 500         | 12.2 c| 10.8–13.6               | 9.3 (10)| 3.9                | 18.3 b| 14.9–21.6               | 4.1 (0.31)| 9.3 (10)             |
| Water pepper  | Ethanol | 500         | 12.0 b| 9.7–14.2                | 14.1 (10)| 4.0                | 19.8 b| 14.7–24.7               | 3.5 (0.26)| 14.1 (10)            |
|               | Acetone | 500         | 15.2 c| 12.8–17.6               | 17.5 (10)| 3.1                | 20.0 b| 16.5–23.3               | 4.3 (0.33)| 17.5 (10)            |
| Banyan        | Ethanol | 500         | 13.8 c| 11.0–16.3               | 20.7 (10)| 3.5                | 17.5 b| 13.7–21.1               | 3.8 (0.30)| 20.7 (10)            |
|               | Acetone | 500         | 10.2 b| 8.0–13.2                | 20.3 (10)| 4.7                | 16.4 b| 11.6–20.7               | 3.0 (0.24)| 20.3 (10)            |
| Crown flower  | Ethanol | 500         | 10.4 b| 7.2–13.1                | 22.6 (10)| 4.6                | 16.2 b| 10.8–21.0               | 2.7 (0.23)| 22.6 (10)            |
|               | Acetone | 500         | 25.1 c| 18.0–31.9               | 67.5 (10)| 1.9                | 26.8 b| 22.3–31.0               | 4.7 (0.35)| 67.5 (10)            |
| Control       |        | 500         | 47.6 d| 44.3–51.5               | 47.6 c| 1.9                | 47.6 c| 44.3–51.5               | 4.0 (0.38)| 1.9 (10)             |

LT₅₀ values in a column followed by different letters are statistically significant (Robertson et al. 2007).
in the form of an emulsifiable concentrate (Kambrekar & Awaknavar 2004). Thus, the different results of toxicity tests in this study might be caused by different toxic compounds being extracted.

The synergistic effects of neem (ethanol) with crown flower (acetone), oleander (acetone), and sweet sop (acetone) extracts to A. dispersus were significant (Table 4). Sakthivel et al. (2011) found a synergistic effect of dimethoate (0.05%) with neem oil (3%), with this combination providing mortality in eggs (81%) and nymphs and adults (94%) of A. dispersus. The plant extracts tested probably displayed different modes of action against A. dispersus. Azadirachtin (the major ingredient of neem insecticide) alone has been shown to have various modes of action including direct effects by inhibiting cell division and protein synthesis and indirect effects by blocking hormone release (Mordue [Luntz] & Nisbet 2000). Mixtures of plant extracts with different modes of action can enhance synergism and delay the development of resistance in A. dispersus. A mixture of different active compounds was suggested for Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) control to reduce the development of insecticide resistance (Ismian 2000; Dubey & Sundararaj 2004). Myzus persicae (Sulzer) (Hemiptera: Aphididae) developed resistance to pure azadirachtin but not to neem seed extract; the different constituents in the seed extract (i.e., azadirachtin, salamin, nimbin, and their respective analogues) might have different modes of action or different target sites in the aphid (Feng & Isman 1995). Prakash & Rao (1997) did an extensive revision of plants containing secondary compounds active against insects. These authors reported 866 plant products with insecticidal, repellent, antifeedant, or insect growth regulator activity. Larew & Locke (1990) proposed that botanical insecticides may involve the removal of insects’ cuticle wax, physical action, repellency, or cell membrane disruption. Valladares et al. (2003) examined the antifeeding activity of an extract of senescent leaves of Melia azadirachta L. (Meliaceae) on 9 insect species and found leaf extract strongly deterred feeding and increased mortality. Our results also show the highest repellency rate in neem (ethanol) plant extract (Table 5).

In conclusion, this study indicates that neem (ethanol), 5-leaved chaste tree (ethanol), sweet sop (acetone), water pepper (acetone), banyan (ethanol), and crown flower (acetone) extracts were the most effective extracts tested against A. dispersus. All the plant extract mixtures tested showed synergism against A. dispersus. These results should promote the development of new products suitable for controlling A. dispersus in organic guava orchards. Future studies are needed to screen active components and develop effective formulations for use in the field.

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