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-leaved 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) extracts at 500 mg/L at 12 h after the spray. For the dry film method, the highest mortality (100%) of adults was also recorded for neem (ethanol) extract (500 mg/L) 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-leaved chaste tree, sweet sop, 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

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 aspersión tópical y de contacto con una película seca 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 tópicales. 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

The spiraling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), is a polyphagous pest attacking agronomic, horticultural, and ornamental crops in tropical and subtropical regions of the world (Bryne et al. 1990; Aiswariaya et al. 2007). Recently, *A. dispersus* has become the most serious pest of guava, *Psidium guajava* (Myrtales), orchards in Bangladesh and India (Kajita & Alam 1996; Aiswariaya et al. 2007), having been introduced through the movement of plant materials from other countries (Kajita & Alam 1996). The pest has also been reported in Brazil, Ecuador, Peru, the Philippines, Fiji, Indonesia, the Maldives, the Mariana Islands, and the Canary Islands on a variety of hosts (Waterhouse & Norris 1989; Nasruddin & Stocks 2014).

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 madhobilota (*Hiptage benghalensis* [L.] Kurz; Malpighiaceae) in Bangladesh (Alim et al. 2014).

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Chemical insecticides are partially effective against spiraling whitefly (Liu et al. 2007) but do not always provide effective control due to the presence of wax covering immature stages (James 2003). Because both nymphs and adults of *A. dispersus* feed on the lower surfaces of leaves, it is difficult to achieve effective coverage by spraying contact insecticides. Furthermore, the use of chemical pesticides may promote resistance in the pest and pollute the environment (Amjad et al. 2009). Thus, there is a need for alternative approaches to replace or supplement the current chemical-based pest management practices in guava orchards (Dubey & Sundararaj 2004).

Plant extracts are potential alternatives due to their high toxicity to insects, low development cost, and general safety to people (Isman 2000).To promote the use of such materials, we examined the toxicity of 8 plant extracts, neem (*Azadirachta indica* A. Juss; Meliaceae), 5-leaved chaste tree (*Vitex negundo* L.; Lamiaceae), sweet sop (*Annona squamosa* L.; Annonaceae), oleander (*Nerium indicum* L.; Apocynaceae), China box (*Buxus sinica* [Rehder & E.H. Wilson] M. Cheng; Buxaceae), water pepper (*Polygonum hydropiper* L.; Polygonaceae), banyan (*Ficus benghalensis* L.; Moraceae), and crown flower (*Calotropis gigantea* [L.] W. T. Aiton; Apocynaceae), and mixtures of these that traditionally have been used in Bangladesh and India (Rajashekar et al. 2012; Ahad et al. 2015) to suppress *A. dispersus*.

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. *Aleurodicus 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 *Aleurodicus dispersus* adults by topical spray.

Common name	Scientific name (family)	Solvent	Dose (mg/L)	Mortality (± SE, %) at hours of application[a]		
				6	12	18
Neem/Margosa	*Azadirachta indica* (Meliaceae)	Ethanol	500	100 a	—	—
			400	77.8 (0.23) a–h	91.1 (2.88) ab	93.3(3.36) ab
		Acetone	500	51.1 (0.56) e–n	73.8 (1.85) abc	100 a
			400	75.6 (0.09) a–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
		Acetone	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	88.9 (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
		Acetone	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
		Acetone	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
		Acetone	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
		Acetone	500	95.6 (0.09) ab	100 a	—
			400	77.8 (0.23) a–h	88.9 (2.80) ab	93.3 (3.36) ab
Banyan	*Ficus benghalensis* (Moraceae)	Ethanol	500	91.1 (0.09) a–d	100 a	—
			400	75.6 (0.09) a–i	86.7 (2.84) ab	100 a
		Acetone	500	95.6 (0.17) ab	100 a	—
			400	77.8 (0.23) a–h	86.7 (2.97) ab	95.6 (3.61) ab
Crown flower	*Calotropis gigantea* (Apocynaceae)	Ethanol	500	84.5 (0.09) a–f	100 a	—
			400	66.7 (0.15) a–k	91.1 (2.63) ab	100 a
		Acetone	500	42.2 (0.23) h–n	66.7 (1.55) a–d	86.7 (2.45) ab
			400	26.7 (0.15) o	60.0 (0.91) a–d	75.6 (2.28) ab
Control				0 o	6.7 (0.15) e	11.1 (0.09) c

[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-immobilization 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: (LD_{50} of toxicant alone / LD_{50} 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 (%) = (N_c − 50) × 2, where N_c = 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 (Zar 2010). Data on lethal effects, Chi-squared values, and LT_{50} 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. LD_{50} 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. DISPERSUS* 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. DISPERSUS* ADULTS

The effects of 3 different mixtures, neem (ethanol) plus crown flower (acetone), neem (ethanol) plus oleander (acetone), and neem (ethanol) plus sweet sop (ethanol) 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. DISPERSUS* 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.

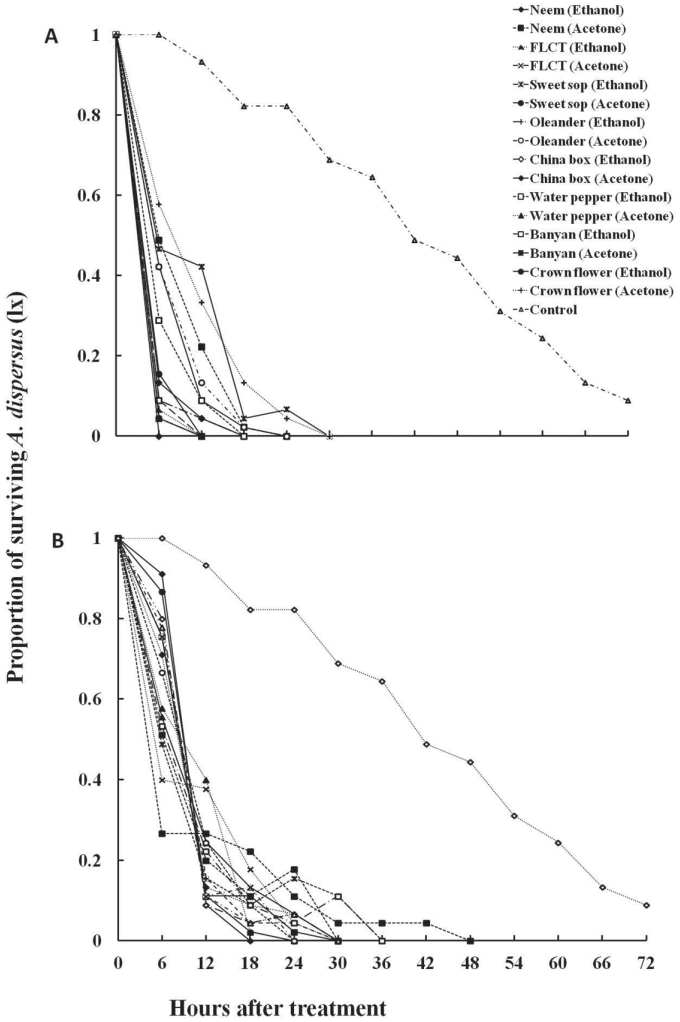

Fig. 1. Survival of *Aleurodicus dispersus* adults exposed to the plant extracts tested by topical spray: 500 mg/L (A), 400 mg/L (B).

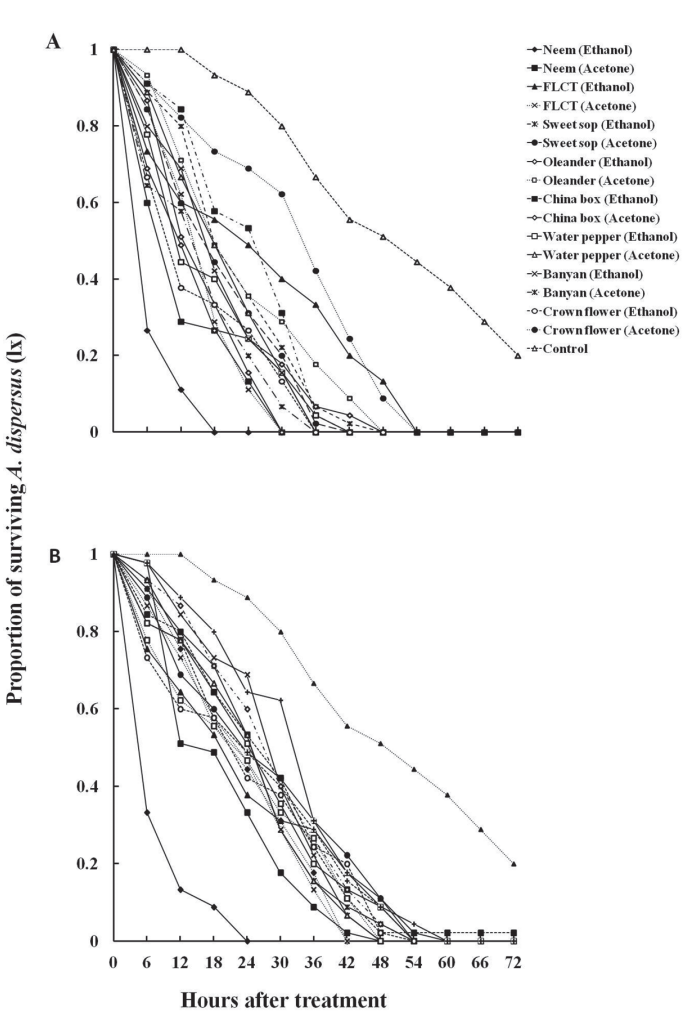

Fig. 2. Survival of *Aleurodicus dispersus* adults exposed to the plant extracts tested by the dry film method: 500 mg/L (A), 400 mg/L (B).

Table 2. Statistical comparison of LT_{50} values of plant extracts against *Aleurodicus dispersus* adults tested by topical spray (n = 45).

		Dose (mg/L)									
		500					400				
Plant extract	Solvent	LT_{50} (h)	95% confidence interval	Slope (± SE)	χ^2 (df)	Toxicity index (TI)	LT_{50} (h)	95% confidence interval	Slope (± SE)	χ^2 (df)	Toxicity index (TI)
Neem	Ethanol[a]	—[c]	—	—	—	44.1	—[c]	—	—	—	44.1
	Acetone	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
Five-leaved chaste tree	Ethanol[b]	—[c]	—	—	—	44.1	5.7 a	4.1 -7.2	3.0 (0.41)	3.8 (10)	7.7
	Acetone	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	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[b]	—[c]	—	—	—	44.1	3.0 a	0.4–4.5	3.6 (1.24)	0.3 (10)	14.8
Oleander	Ethanol	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	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
China box	Ethanol	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	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	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[b]	—[c]	—	—	—	44.1	3.4 a	1.5–5.0	2.5 (0.50)	4.2 (10)	13.2
Banyan	Ethanol[b]	—[c]	—	—	—	44.1	4.0 a	2.2–5.4	3.2 (0.64)	5.4 (10)	11.1
	Acetone[b]	—[c]	—	—	—	44.1	3.5 a	1.7–5.1	2.7 (0.52)	4.0 (10)	12.6
Crown flower	Ethanol[b]	—[c]	—	—	—	44.1	4.8 a	3.2–6.0	4.0 (0.80)	1.7 (10)	9.1
	Acetone	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		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).
[a]Extract killed all *A. dispersus* adults within 6 h after treatment.
[b]Extract killed all *A. dispersus* adults within 12 h after treatment.
[c]By 6 and 12 h after treatments, all insects had died, and analysis did not give LT_{50} values.

Table 3. Statistical comparison of LT_{50} values of plant extracts against *Aleurodicus dispersus* adults tested by dry film (n = 45).

		Dose (mg/L)									
		500					400				
Plant extract	Solvent	LT_{50}	95% confidence interval	Slope (± SE)	χ^2 (df)	Toxicity index (TI)	LT_{50}	95% confidence interval	Slope (± SE)	χ^2 (df)	Toxicity index (TI)
Neem	Ethanol	4.2 a	2.5–5.5	3.4 (0.69)	3.5 (10)	11.3	4.7 a	3.0–6.1	3.0 (0.46)	3.5 (10)	10.3
	Acetone	8.2 b	6.1–10.2	3.2 (0.32)	13.7 (10)	5.8	15.8 b	13.4–18.1	4.1 (0.34)	13.7 (10)	3.0
Five-leaved chaste tree	Ethanol	16.8 c	11.1–21.9	3.2 (0.32)	38.2 (10)	2.8	16.1 b	12.0–19.9	2.7 (0.22)	38.2 (10)	3.0
	Acetone	12.2 c	10.7–13.8	3.8 (0.29)	10.2 (10)	3.9	23.5 b	19.3–27.4	5.1 (0.38)	10.2 (10)	2.0
Sweet sop	Ethanol	16.9 c	14.1–19.5	4.6 (0.40)	19.1 (10)	2.8	20.3 b	16.5–23.9	4.1 (0.30)	19.1 (10)	2.4
	Acetone	14.3 c	11.6–16.7	3.8 (0.29)	18.0 (10)	3.3	20.0 b	15.7–24.0	3.2 (0.25)	18.0 (10)	2.4
Oleander	Ethanol	10.9 b	8.6–13.0	3.8 (0.29)	11.5 (10)	4.4	19.1 b	14.5–23.5	3.2 (0.25)	11.5 (10)	2.5
	Acetone	17.7 c	15.3–20.0	2.9 (0.26)	12.5 (10)	2.7	19.0 b	14.5–23.3	3.3 (0.25)	12.5 (10)	2.5
China box	Ethanol	19.1 c	14.6–23.3	4.5 (0.34)	45.3 (10)	2.5	22.8 b	18.1–27.2	4.3 (0.31)	45.3 (10)	2.1
	Acetone	12.2 c	10.8–13.6	4.4 (0.40)	9.3 (10)	3.9	18.3 b	14.9–21.6	4.1 (0.31)	9.3 (10)	2.6
Water pepper	Ethanol	12.0 b	9.7–14.2	3.3 (0.28)	14.1 (10)	4.0	19.8 b	14.7–24.7	3.5 (0.26)	14.1(10)	2.4
	Acetone	15.2 c	12.8–17.6	4.2 (0.34)	17.5 (10)	3.1	20.0 b	16.5–23.3	4.3 (0.33)	17.5 (10)	2.4
Banyan	Ethanol	13.8 c	11.0–16.3	3.8 (0.30)	20.7 (10)	3.5	17.5 b	13.7–21.1	3.8 (0.30)	20.7 (10)	2.7
	Acetone	10.2 b	8.0–13.2	3.3 (0.29)	20.3 (10)	4.7	16.4 b	11.6–20.7	3.0 (0.24)	20.3(10)	2.9
Crown flower	Ethanol	10.4 b	7.2–13.1	3.0 (0.27)	22.6 (10)	4.6	16.2 b	10.8–21.0	2.7 (0.23)	22.6 (10)	3.0
	Acetone	25.1 c	18.0–31.9	3.8 (0.28)	67.5 (10)	1.9	26.8 b	22.3–31.0	4.7 (0.35)	67.5 (10)	1.8
Control		47.6 d	44.3–51.5	4.0 (0.38)	1.9 (10)	1.0	47.6 c	44.3–51.5	4.0 (0.38)	1.9 (10)	1.0

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

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 ethanol extracts of *Celosia argentea* L. (Amaranthaceae) and *Eupatorium odoratum* L. (Asteraceae) had high activity against *A. dispersus*, with LC_{50} values of 753.4 and 999.8 mg/L, respectively.

Our results indicated that neem, 5-leaved chaste tree, sweet sop, oleander, China box, water pepper, banyan, and crown flower extracts at 500 mg/L all exhibited significant insecticidal action against *A. dispersus* in the topical spray assay. Among the 8 plant species, the highest adult mortality was found with neem (ethanol) extracts irrespective of assay method (Tables 1 and 2), although acute toxicity of neem extract was higher when it was extracted in ethanol than when extracted in acetone. Ethanol is a better solvent for extracting more of the toxic component from neem leaves (Sharma et al. 2010). The next most 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, Neemark® (West Coast Herbochem Ltd. Mumbai, India) retained its toxicity (from an initial mortality of 55% to 45% on day 15) better than NeemAzal® T/S (E.I.D. Parry, India, Ltd.) (45% to 25% mortality); Neemark® is neem leaf extract 2% w/w and NeemAzal® contains 10 g/L azadirachtin A

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

Plant extract	Ratio	LD_{50} (mg/L)	95% confidence interval	Regression equation	χ^2(df)	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)	173.0
	1:2	127.2	27.9–581.0	$y = 0.74 + 2.00x$	8.4 (1)	182.8
	1:3	90.6	10.0–817.0	$y = 1.05 + 2.01x$	1.2 (1)	256.8
Neem (ethanol) + oleander (acetone)	1:1	227.8	170.0–305.2	$y = -8.57 + 5.75x$	0.1 (1)	102.0
	1:2	211.5	130.0–344.0	$y = -3.05 + 3.46x$	0.9 (1)	109.9
	1:3	192.0	101.8–362.2	$y = -2.08 + 3.10x$	0.0 (1)	121.1
Neem (ethanol) + sweet sop (ethanol)	1:1	165.8	67.9–404.5	$y = -0.89 + 2.65x$	7.7 (1)	140.2
	1:2	142.7	41.5–488.9	$y = 0.14 + 2.25x$	0.2 (1)	163.0
	1:3	132.0	37.7–461.7	$y = -0.68 + 2.68x$	1.1 (1)	176.2

Table 5. Repellency rates of tested plant extracts against *Aleurodicus dispersus* adults at different hours after treatment.

		Repellency (%) at different hours after treatment[a]				
Plant extract	Solvent	3	6	24	Mean repellency	Repellency class
Neem	Ethanol	88.8 a	91.1 ab	100 a	93.3	V
	Acetone	37.8 bc	62.2 c	71.1 de	57.0	III
Five-leaved chaste tree	Ethanol	31.1cd	48.9 d	48.9 h	43.0	III
	Acetone	42.2 cd	100 a	66.7 def	69.6	IV
Sweet sop	Ethanol	57.8 bc	62.2 c	77.8 cd	65.9	IV
	Acetone	22.2 c	31.1 e	53.3 gh	35.6	II
Oleander	Ethanol	26.7 c	31.1 e	86.8 bc	48.2	III
	Acetone	37.7 cd	48.9 d	57.8 fgh	48.2	III
China box	Ethanol	20.0 c	31.1 e	84.4 bc	45.2	III
	Acetone	33.3 cd	48.9 d	88.9 abc	57.0	III
Water pepper	Ethanol	73.3 ab	86.7 b	91.1 ab	83.7	V
	Acetone	80.0 ab	86.7 b	86.7 bc	84.5	V
Banyan	Ethanol	15.6 c	51.1d	62.2 efg	43.3	III
	Acetone	31.1 cd	51.1 d	60.0 efgh	47.4	III
Crown flower	Ethanol	73.3 ab	86.7 b	91.1 ab	83.7	V
	Acetone	73.3 ab	57.8 b	86.7 bc	72.6	IV

[a]Means in a column followed by different letters indicate significant differences from the Tukey test ($\alpha = 0.05$).

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 (ethanol) 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 could 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 (Isman 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, salanin, 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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