PEST MANAGEMENT

Control of Silverleaf Whitefly, Cotton Aphid and Kanzawa Spider Mite with Oil and Extracts from Seeds of Sugar Apple

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Controle da Mosca-Branca, do Pulgão do Algodoeiro e do Ácaro de Kanzawa com Óleo e Extratos de Sementes de Fruta-do-Conde

RESUMO - É crescente a necessidade de desenvolvimento de métodos alternativos para o manejo de pragas com o aumento da consciência pública sobre os efeitos adversos de pesticidas à saúde humana e ao ambiente. O objetivo principal deste trabalho foi o de avaliar o óleo de sementes de fruta-do-conde (*Annona squamosa*), uma fruta tropical comestível, para o controle de pragas. O óleo prensado de sementes foi tão eficiente quanto o pesticida recomendado para controle da mosca-branca *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae) infestando folhas de tomate em casa-de-vegetação, com a vantagem de não apresentar fitotoxicidade. Em observações ao microscópio eletrônico de varredura, o óleo de semente induziu ao ressecamento das ninfas e o seu desprendimento da superfície da folha. O óleo de sementes de fruta-do-conde também foi eficaz no controle do pulgão do algodoeiro, *Aphis gossypii* Glover (Homoptera: Aphididae), infestando folhas de melão, e do ácaro de Kanzawa, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae), em folhas de soja. Este estudo revelou a possibilidade de utilizar o óleo de sementes de fruta-do-conde, um sub-produto agrícola, como produto de largo espectro de ação mas seguro ao ambiente e à saúde humana, em programas de manejo de pragas agrícolas.

PALAVRAS-CHAVE: Manejo de pragas, controle alternativo, planta inseticida, *Annona*

ABSTRACT - Development of alternative methods for pest management is needed with the increased concern for adverse effects of pesticides for human health and the environment. The main goal of our study was to test the oil from seeds of sugar apple (*Annona squamosa*), an edible tropical fruit for pest control. The oil pressed out of seeds was as effective in controlling the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae), infesting leaves of tomato plants in greenhouse conditions as the recommended insecticide, with the advantage of not being phytotoxic. When observed with a scanning electron microscope, the seed oil caused whitefly nymphs to shrink and detach from the leaf surface. Sugar apple seed oil was also very effective in controlling the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), on melon leaves and the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae), on soybean leaves. The study revealed the possibility of developing the oil from sugar apple seeds, an agricultural waste, into a broad spectrum product friendly to the environment and human health for crop pest management.

KEY WORDS: Pest management, non-pesticide control, *Annona*

Intensive use of synthetic pesticides to control agricultural pests has created numerous problems such as poisoning of farm workers, consumers, and wildlife, resistance to pesticides in pest populations and negative environmental impacts. These adverse effects have provided the impetus for the development of alternatives to chemical pesticides for pest management. Natural products derived from plants have been shown to be promising alternatives (Cropping & Menn 2000, Isman 2006, Cropping & Duke 2007). A number of vegetable and essential oils extracted from various spices and herbs have been shown to contain insecticidal properties (Singh & Upadhyay 1993, Isman 2000). Since edible fruit seeds also contain oils and there are so many kinds of fruits in tropic and subtropic areas of Taiwan, a project was initiated to test such oils for pest control.

The silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, previously referred to as *Bemisia tabaci* biotype B was used as the test organism in this study because it is a devastating pest of numerous crops in many parts of the world (Lin *et al* 1997, Cohen *et al* 1998, Oliveira *et al* 2001) and insecticides have been heavily used for its control (Palumbo *et al* 2001). During the screening tests, oil pressed out of seeds of sugar apple (*Annona squamosa*) was found to be very effective in causing mortality of silverleaf whitefly nymphs, while its aqueous extract was ineffective and the ethanolic extract was only slightly effective. Since the cotton aphid, *Aphis gossypii* Glover (Conway *et al* 2003), and the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Gotoh & Gomi 2003), are also important pests of many agricultural crops and pesticides have been frequently applied for their control, they were also tested. It was found that oil from sugar apple seeds was also very effective in reducing the survival rate of these two pests. Details of the study are reported herein.

Material and Methods

Preparation of seed extracts. Seeds of sugar apple were collected from the waste of sugar apple ice cream processing factory in Taitung, the major sugar apple production area in Taiwan. Collected seeds were washed with water to remove contaminating materials and air dried. Oil was pressed out of the seeds with an oil extractor (Shang Jer Industries Co., Chiayi, Taiwan). Approximately 180 ml of oil were extracted from 1 kg of seeds. For emulsification, a 250 ml flask containing 100 ml oil and 15 ml Tween 80 was vigorously shaken by hand 20 times. The mixture was diluted with distilled water to make 0.5, 0.25 or 0.125% (v/v) of oil testing solutions for pest control.

Aqueous extract was prepared by grinding 100 g of sugar apple seeds with 300 ml of distilled water in a waring blender at high speed for 3 min. The mixture was left on the bench for two days, passed through a juice extractor and centrifuged at 2700 *g* for 30 min to produce a clear extract. Approximately 1800 ml of aqueous extract were obtained from 1 kg of seeds, about 10 times of oil pressed out of the same amount of seeds. The extract was diluted with distilled water to make 1.5, 0.75 or 0.375% (v/v) extract solutions for pest control tests. The percentage was calculated based on a 10-fold concentrate of the original for easy direct comparison with seed oil.

Ethanolic extract was similarly prepared by grinding 100 g of sugar apple seeds with 300 ml of 50% ethanol in a waring blender at high speed for 3 min, and processed as before. The amount of ethanolic extract generated per kg was about the same as the aqueous extract. The extract was diluted with distilled water as earlier described.

Pest sources. Naturally silverleaf whitefly-infested tomato (*Lycopersicum esculentum*) plants grown in pots in the greenhouse were used for the study when they reached the stage with seven to eight fully expanded leaves. Cotton aphids were maintained on leaves of melon (*Cucumis melo*) plants grown in a screen cage (150 × 60 × 180 cm), while Kanzawa spider mites were maintained on leaves of soybean (*Glycine max*) plants grown in a growth chamber at 25ºC.

Spray treatments. Naturally silverleaf whitefly-infested tomato leaves were sprayed to runoff. For comparison, plants were similarly sprayed with β-cyfluthrin + imidacloprid, an insecticide formula recommended for silverleaf whitefly and cotton aphid control (Fue & Wang 2004), at the concentration of 0.05% (w/v) as suggested by the manufacturer (Bayer Crop Science Taiwan Co., Taipei, Taiwan). The same procedure was used to test the effect of aqueous and ethanolic extracts on mortality of silverleaf whitefly at the concentration of 1.5, 0.75 or 0.375%. Water was used as the control for the former and 0.25% ethanol for the latter. Nymph mortality was determined by the body movement upon being slightly touched by a fine brush hair under a dissecting microscope at 20x magnification, on days 3, 5, 7 and 10 after spray. Three plants were used for each treatment, three leaves per treatment were removed for observation each time, and the experiment was repeated three times.

For testing the effect of sugar apple seeds emulsified oil on aphid mortality, melon plants each with seven to eight fully expanded leaves grown in aphid-free screen cage were used. Leaf infestation was carried out by placing upside down a piece (ca. 2 × 2 cm) of a melon leaf containing 10 adult cotton aphids from the aphid-source plant on a test melon leaf. The leaf piece was removed after movement of aphids to the test leaf was completed in 2h. Aphids were allowed to multiply for three to four days before being sprayed with emulsified oil, Tween 80 (control) or β-cyfluthrin + imidacloprid for comparison as earlier described. Aphid mortality was determined by observing the body shape under a dissecting microscope at 20x magnification. After death, aphids shrank and became thin. Three plants were used for each treatment, three leaves per treatment were removed for observation at each recording time, and the experiment was repeated three times.

For testing the effects of the sugar apple seeds emulsified oil on the mortality of the Kanzawa spider mite, soybean plants each with four fully expanded leaves grown in the mite-free growth chamber were used. Detached leaves were placed individually on a 9 × 9 cm thick cloth squares containing 12 ml distilled water in a large petri dish (15 × 2 cm). Leaves remained fresh and alive under such conditions for two weeks in the growth chamber. Thirty Kanzawa spider mites were transferred from the mite-source plant to each leaf with a fine calligraphic brush. For comparisons, detached leaves were sprayed similarly with etoxazole (Fue & Wang 2004) at the concentration of 0.03% (w/v) as suggested by the manufacturer (Sumitomo Chemical Taiwan Co., Taipei, Taiwan). Mite mortality was determined by the body movement upon contact by a fine hair brush under a dissecting microscope at 20x magnification. Fifteen plates were used for each treatment, three plates per treatment were observed at each recording time, and the experiment was repeated three times. The Tukey's significant difference test was used to analyze all data presented.

Scanning electron microscopy. For observations with a scanning electron microscope, triangular pieces (ca. 0.2 cm each side) of leaf, each containing a nymph of silverleaf whitefly, were excised with a surgical scalpel under the dissecting microscope at 30x magnification, and adhered onto aluminum specimen mounts with double adhesive tape, immediately frozen in liquid nitrogen and dried in a vacuum chamber. Samples were then placed on the cryo-stage and viewed with a JSM-6330F field emission scanning electron microscope (Jeol, Tokyo, Japan) (Ko *et al* 2003).

Results

Effect of sugar apple oil on the silverleaf whitefly. Sugar apple seeds emulsified oil at 0.5% caused 55.8% mortality of silverleaf whitefly nymphs in five days when sprayed on infested tomato leaves in the greenhouse (Table 1), against only 1.5% mortality in the control. Ten days after the spray, the mortality rates increased from 8.0% in the control to more than 90% when 0.5 or 0.25% oil treatments were used. The treatment with 0.125% oil also increased the mortality rate to 54.1%, more than 50% during the same period of time. Sugar apple seed oil was not phytotoxic to tomato plants even at the concentration of 0.5%. The mortality rates caused by β-cyfluthrin + imidacloprid were similar to those caused by 0.5 or 0.25% of sugar apple seed oil during the test period (Table 1).

After being sprayed with sugar apple seed oil, whitefly nymph body became shrunken and detached from the tomato leaf surface when observed with the scanning electron microscope (Fig 1A), but remained adhered to the surface of tomato leaves on control plants (Fig 1B).

Effect of aqueous and ethanolic extracts of sugar apple seeds on the silverleaf whitefly. Spraying aqueous extract on naturally infested tomato plants in the greenhouse did not affect mortality rates of silverleaf whitefly nymphs on tomato leaves. Ten days after the spray, the mortality rate of whitefly nymphs on tomato leaves treated with 1.5% extract was 39.7%, which was not significantly different from 29.0% on control leaves (Table 2).

Ethanolic extract of sugar apple seeds was slightly toxic to nymphs of silverleaf whitefly at 1.5%, but not at 0.375% or 0.75% (Table 3). Ethanolic extract at 1.5% increased the mortality rate of whitefly nymphs from 21.4% on the control leaves to 45.6% on the treated leaves, five days after

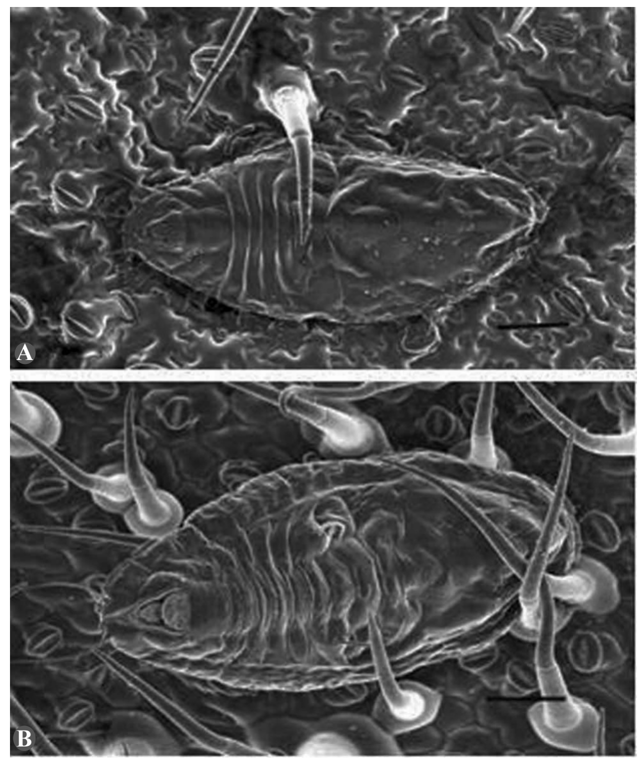

Fig 1 Scanning electron micrographs of nymphs of silverleaf whitefly on tomato leaves two days after spray treatment. (A) Sprayed with 0.5% sugar apple seed oil emulsified with Tween 80 (B) Sprayed with Tween 80 as control. Scale bar = 50 µm.

Table 1 Effect of oil pressed out of sugar apple seeds on the mortality of silverleaf whitefly nymphs infesting tomato leaves in the greenhouse.

Treatment	Mortality (%)[1], days after application			
	3	5	7	10
Seed oil[2]				
0.5%	22.0 ± 12.45 AB	55.8 ± 26.50 A	81.0 ± 11.86 A	98.3 ± 3.04 A
0.25%	23.8 ± 10.24 AB	30.3 ± 14.37 AB	66.2 ± 12.78 AB	95.6 ± 4.56 A
0.125%	12.5 ± 9.01 AB	35.5± 18.22 AB	45.6 ± 11.48 AB	54.1± 12.90 B
β- cyfluthrin + imidacloprid 0.05%	37.4 ± 16.34 A	53.1 ± 21.71 A	73.3 ± 9.64AB	92.7 ± 1.08 A
Control	1.6 ± 2.75 B	1.5 ± 1.59 B	1.5 ± 1.59 C	8.0 ± 2.36 C

[1]Three plants were used for each treatment and three leaves per treatment were removed for observation at each recording time. The number of nymphs on each leaf ranged from 16 to 178 with an average of 67.

[2]Approximately 0.18 ml oil was pressed out of 1 g seeds.

Values in the same column followed by the same letter are not significantly different by Tukey's significance test (α = 0.05).

Author, the letters in red in this table should be only A, no? If the mortality is different of control and if 53.1% mortality in the same column is A.

Table 2 Effect of aqueous extract of sugar apple seeds on the mortality of silverleaf whitefly nymphs infesting tomato leaves in the greenhouse.

Treatment	Mortality (%)[1], days after application			
	3	5	7	10
Aqueous extract[2]				
1.5%	14.1 ± 17.25 A	37.6 ± 32.64 A	39.7 ± 29.04 A	39.7 ± 29.04A
0.75%	7.9 ± 7.05 A	17.2 ± 10.75 A	28.1 ± 17.78A	31.8 ± 22.37 A
0.375%	19.9 ± 17.51 A	28.0 ± 22.37A	43.1 ± 14.57A	46.8 ± 16.44 A
Control	12.7 ± 8.39 A	21.4 ± 8.66A	26.3 ± 14.69 A	29.0 ± 12.22 A

[1]Three plants were used for each treatment and three leaves per treatment were removed for observation at each recording time. The number of nymphs on each leaf ranged from 11 to 161 with an average of 70.

[2]Approximately 1.8 ml aqueous extract was obtained from 1 g seed. The percentage was calculated based on a 10-fold concentrate of the original extract for easy direct comparison with seed oil.

Values in the same column followed by the same letter are not significantly different by Tukey's significant difference test ($\alpha = 0.05$).

treatment. After 10 days, the mortality rate was 69.7% on the treated leaves and 29.0% on the control leaves. Although the mortality rate of whitefly nymphs on leaves sprayed with ethanolic extract at 0.375% or 0.75% was higher than that in control, no differences were observed after 10 days.

The variation in mortality rates of the silverleaf whitefly observed in the control treatments when testing the different extracts of the sugar apple oil seed occurred due to the collection of whiteflies at different times from the greenhouse (Tables 1-3).

Effect of sugar apple seed oil on the cotton aphid. The cotton aphid was also effectively controlled three days after being sprayed with 0.5% emulsified sugar apple seed oil (Table 4) or at 0.25%, five days after spray. Although the oil at 0.125% was able to kill 83.5% of aphids on the 3rd day, the mortality rate decreased to 65.0% on the 5th day due to the propagation of the surviving aphids. By the 10th day, the number of aphids killed was almost completely compensated with the new born ones (Table 4). The insecticide formula, β-cyfluthrin + imidacloprid, used for comparison also killed all tested aphids within three days.

Effect of sugar apple seed oil on the Kanzawa spider mite. Tests conducted on the detached leaves in the growth chamber showed that sugar apple seed oil was very effective in killing the Kanzawa spider mite, with more than 80% mortality three days after application of 0.5% or 0.25% seed oil. The mortality rates increased to more than 90% in five days, and by the 10th day all the spider mites tested were killed (Table 5). Even at 0.125%, the oil was able to cause 100% mortality of spider mites in 10 days. In the control, the mortality rate was 26.9% after 10 days. The miticide etoxazole used for comparison killed 96.7% of spider mites in 10 days.

Discussion

Many plant species of the Annonaceae family have been used in folklore as antitumor agents, emetics, etc (Rupprecht

Table 3 Effect of ethanol extract of sugar apple seeds on the mortality of silverleaf whitefly nymphs infesting tomato leaves in the greenhouse.

Treatment	Mortality (%)[1], days after application			
	3	5	7	10
Ethanolic extract[2]				
1.5%	23.6 ± 8.92 A	45.6 ± 13.02 A	60.0 ± 16.52A	69.7 ± 15.89A
0.75%	17.4 ± 4.26 A	22.1 ± 4.21 B	34.7 ± 7.55 AB	50.5 ± 6.37 AB
0.375%	12.2 ± 5.72 A	18.7 ± 5.92 B	25.9 ± 7.28 B	56.2 ± 5.49 AB
Control	12.7 ± 8.39 A	21.4 ± 8.66 B	26.3 ± 14.69 B	29.0 ± 12.22 B

[1]Three plants were used for each treatment and three leaves per treatment were removed for observation at each recording time. The number of nymphs on each leaf ranged from 10 to 163 with an average of 40.

[2]Approximately 1.8 ml alcoholic extract was obtained from 1 g seeds. The percentage was calculated based on a 10-fold concentrate of the original extract for easy direct comparison with seed oil.

Values in the same column followed by the same letter are not significantly different by Tukey's significant difference test ($\alpha = 0.05$).

Table 4 Effect of oil pressed out of sugar apple seeds on the mortality of the cotton aphid feeding on melon leaves in the screen cage.

Treatment	Mortality (%)[1], days after application			
	3	5	7	10
Seed oil[2]				
0.5%	100 A	100 A	100 A	100 A
0.25%	95.7 ± 7.47 A	100 A	100 A	100 A
0.125%	83.5 ± 13.03 A	65.0 ± 23.50 B	57.4 ± 13.54 B	3.7 ± 1.63 B[3]
β- cyfluthrin + imidacloprid 0.05%	100 A	100 A	100 A	100 A
Control	0 B	0 C	0 C	0 B

[1]Three plants were used for each treatment and three leaves per treatment were removed for observation at each recording time. The number of adult aphids on each leaf before treatment ranged from 34 to 115 with an average of 76.

[2]Approximately 0.18 ml oil was pressed out of 1 g seeds.

[3]Mortality rate dropped due to propagation of surviving aphids.

Values in the same column followed by the same letter are not significantly different by Tukey's significant difference test ($\alpha = 0.05$).

et al 1990). For this reason, a number of bioactive compounds from these plants have been characterized (Rupprecht *et al* 1990, Xu 2001). Among these compounds, annonins and annonacin of acetogenins present insecticidal activity, while anonaine of alkaloid have bactericidal and fungicidal properties (Rupprecht *et al* 1990, Duke 1992, Xu 2001). Many phytochemicals present in stems, leaves, unripe fruit and seeds of sugar apple (*A. squamosa*) were also identified (Xu 2001). Annonins from this species also present insecticidal properties (Londershausen *et al* 1991). None of the Annonaceous compounds or extracts were reported as miticidal (Xu 2001). Very few species of insects have been tested for their sensitivity to identified compounds or extracts from Annonaceous plants, and none of these were tested for possible control of insect pests in the greenhouse or in the field (Xu 2001).

Our report is the first to show that the silverleaf whitefly, the cotton aphid and the Kanzawa spider mite can be effectively controlled by oil pressed out of sugar apple seeds in the greenhouse. In Chinese medicine, sugar apple seeds are used for expelling parasitic worms from the body (Chiu & Chang 1986, Li & Liu 1998). Since the sugar apple seed oil was also not phytotoxic to tomato plants at the highest concentration used, it may perhaps be relatively safe for use in home gardening and organic farming. Using sugar apple seed oil for pest management also represents an ideal way of managing a special agricultural waste. The potential for developing the sugar apple seed oil into a product friendly to the environment and human health for large-scale application to control agricultural pests remains to be explored.

Aqueous extract and ethanolic extract of sugar apple seeds were reported previously to be effective against diamondback moth (*Plutella xylostella*) (Lepidoptera: Plutellidae) (Leatemia & Isman 2004), and ethyl acetate extract of defatted sugar apple seeds was also found to be toxic to *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Kaeazu *et al* 1989). The effective component in these cases appears to be different from what

Table 5 Effect of oil pressed out of sugar apple seeds on the mortality of the Kanzawa spider mite feeding on soybean leaves in the growth chamber.

Treatment	Mortality (%)[1], days after application			
	3	5	7	10
Seed oil[2]				
0.5%	86.7 ± 6.67 A	96.7 ± 3.33 A	98.9 ± 1.92 A	100 A
0.25%	81.1 ± 6.94 A	92.2 ± 1.92 A	97.8 ± 3.85 A	100 A
0.125%	73.3 ± 6.67 A	87.8 ± 5.09 A	93.3 ± 3.33 A	100 A
Etoxazole 0.03%	78.9 ± 10.72 A	81.1 ± 10.18 A	94.4 ± 1.92A	96.7 ± 3.53 A
Control	0 B	10.0 ± 6.67 B	17.8 ± 5.09 B	26.7 ± 6.67 B

[1]Fifteen plates were used for each treatment and three plates per treatment were observed at each recording time. Each plate contained 30 mites.

[2]Approximately 0.18 ml oil was pressed out of 1 g seeds.

Values in the same column followed by the same letter are not significantly different by Tukey's significant difference test ($\alpha = 0.05$)

we found. In our study, oil pressed out of sugar apple seeds was very toxic to the silverleaf whitefly, but the aqueous and the ethanolic extracts were either non toxic or only slightly toxic. It is also possible that small amounts of active components of the oil were present in the aqueous and ethanolic extracts and that the diamondback moth and the fruit fly were exceptionally sensitive to this component. The active component in the oil will be purified and characterized in the near future.

Nymphs of whiteflies are very tiny and difficult to observe even with a dissecting microscope. Our observation with the scanning electron microscope showed the detachment of the nymph body from the leaf surface after being sprayed with sugar apple seed oil (Fig 1). This indicates the possibility of starvation as a cause of death because whitefly nymphs require intimate contact with the leaf surface for successful feeding. However, the possibility of nymph body detachment as the result of death caused by direct insecticidal activity of sugar apple seed oil cannot be ruled out.

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