

## Use of vegetable oils in the control of the citrus black fly, *Aleurocanthus woglumi* (Hemiptera: Aleyrodidae)

Uso de aceites vegetales para control de la mosca negra de los cítricos, *Aleurocanthus woglumi* (Hemiptera: Aleyrodidae)

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**Abstract:** This research aimed to evaluate different vegetable oils in the control of the citrus black fly, *Aleurocanthus woglumi*. The effect of vegetable oils of soybean (*Glycine max*), corn (*Zea mays*), sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*) and extract of neem (Nim® Rot) was evaluated using four concentrations (0 (control), 0.5, 1.0 and 1.5 mL/L) for each extract, on the fourth-instar nymphs and egg masses of *A. woglumi*. The leaves containing insects were immersed in a 500 mL-beaker containing vegetable oils in their respective concentrations. Data were analyzed according to a randomized design in a factorial scheme (5 x 4) corresponding to vegetable oil and concentration, respectively, followed by means separation with Tukey's test at 5% probability, using the program ASSISTAT 7.5 beta. It was found that all vegetable oils were effective in causing mortality of the insects. The mortality rate of the fourth nymphal stage was above 90% for all vegetable oils used. For the ovicidal activity, cottonseed oil provided 100% egg mortality, whereas corn oil resulted in lower mortality (48.9%). After treatment with the oils, eggs turned to a dark color. The nymphs that hatched often dried out and were therefore not viable. These results indicate the high efficiency of oil applications on the mortality of the citrus black fly.

**Key words:** Citrus. Alternative Products. Integrated Pest Management.

**Resumen:** En esta investigación se buscó evaluar diferentes aceites vegetales en el control de la mosca negra de los cítricos, *Aleurocanthus woglumi*. Se evaluó el efecto de los aceites vegetales de soja (*Glycine max*), maíz (*Zea mays*), girasol (*Helianthus annuus*), algodón (*Gossypium hirsutum*) y extracto de neem (Nim® Rot) usando cuatro concentraciones (0 (control) 0,5; 1,0 y 1,5 mL/L), en ninfas del cuarto instar y posturas de huevos de *A. woglumi*. Las hojas que contenían los insectos se sumergieron en un vaso beaker de 500 mL que contenía aceites vegetales en sus respectivas concentraciones. Se analizaron los datos de acuerdo con un diseño completamente al azar en un esquema factorial (5 x 4) correspondiendo a aceite vegetal y concentración, respectivamente, y la evaluación de las medias se compararon mediante la prueba de Tukey, al 5% de probabilidad, utilizando el programa ASSISTAT 7.5 beta. Se encontró eficiencia de todos los aceites vegetales al ocasionar mortalidad de los insectos. La tasa de mortalidad de ninfas en el cuarto estadio fue superior al 90% para todos los aceites vegetales usados. En cuanto a la acción ovicida, el aceite de algodón proporcionó 100% de mortalidad de los huevos (100%), mientras que el aceite de maíz resultó en mortalidad menor (48,9%). Después de tratamiento con los aceites, los huevos tomaron una coloración oscura. Las ninfas que eclosionaron frecuentemente se secaron y, por lo tanto, no fueron viables. Estos resultados indican la alta eficacia de aplicaciones de aceites en la mortalidad de *A. woglumi*.

**Palabras clave:** Los cítricos. Productos alternativos. Manejo Integrado de Plagas.

### Introduction

The citrus black fly, *Aleurocanthus woglumi* Ashby, 1915 (Hemiptera: Aleyrodidae) is a native pest of Asian origin, but it is widely spread almost all over the world, and it is considered agriculturally important in several countries due the economic loss that it causes (Batista *et al.* 2002). In Brazil, it has been considered a quarantine pest A2, it is an insect pest of potential economic importance, but not widely distributed, and its specific occurrence was reported in 12 Brazilian states resulting in control program by Brazilian government. Approximately 300 host plants of this insect have been reported, being citrus its favorite host for population development (Nguyen and Hamon 1993). Heavy infestations may cause fast deterioration of plants and production decrease (Fasulo and Brooks 1993). The production reduction of severely infested plants may exceed 80% of total production (Yamamoto *et al.* 2008).

Brazil increased its participation in the international market of soybean, coffee, orange, sugar and alcohol ex-

portation among others. The national orange production reached a value of 17.618.450 tons, being the state of São Paulo responsible for 77.4% of the total fruit production in Brazil, followed by the states of Bahia (5.2%) and Sergipe (4.5%) (IBGE 2009). This is the reason why the expansion of this pest in citrus production areas of Brazil is worrying. Initially *A. woglumi* was restricted to the states of Pará, Amapá, Amazonas, Tocantins and Maranhão (Lemos *et al.* 2006), but it has later been detected during phytosanitary inspections performed in citrus orchards from São Paulo state (Pena *et al.* 2008), Goiás (Yamamoto *et al.* 2008), Paraíba (Lopes *et al.* 2009), Ceará (Carneiro 2010), Rio Grande do Norte and Pernambuco (Cavalcante 2010), and recently in Bahia state (Silva 2010).

As the attack by this pest progresses in the agricultural borders, the value of the citrus agribusiness is seriously threatened, despite the economic and social importance that the citrus production has in Brazil there is still a need for research and finding methods of control of the citrus black fly (Lopes 2009). The use of mineral, vegetable or derived

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oils may result in an improvement of control strategies for agricultural pests and associated diseases, and may cause minimum harmful effects upon the populations of natural enemies and other non-target species (Rodrigues *et al.* 2002). In addition, the application of these insecticides may be more environmentally friendly than traditional insecticides.

In Brazil, the use of these alternative pest control strategies to insecticides which are highly toxic to humans and the environment (Fernandes *et al.* 2006), is increasing although poorly applied.

The diversity of the Brazilian flora has shown an immense potential for the production of secondary compounds which may be used as insecticides and/or insects repellents (Fazolin *et al.* 2002). These compounds, such as alkaloids, flavonoids, tannins, quinones, essential oils, saponins, and cardioactive heterosides are produced by the plants for their survival (Cardoso *et al.* 2001). Less than 1% of the Brazilian flora has been searched chemically (Ming 1996). The ethnobotanical knowledge constitutes an excellent starting point for a selection of insecticidal and/or insect repellent activity (Paula 2002).

According to Saito *et al.* (2004), amongst useful substances for the control of insects, it stands out those with insecticidal activity, sterilization activity, repellency and those with feeding inhibiting activity. The influence of the effects and the activity time of solutions depend on the dosage, thus death occurs in the highest dosages and the less intense effects in lower dosages (Roel 2001).

Because of the above mentioned reasons and because there are no studies about the most adequate way of control of this pest in Paraíba, this study aimed to evaluate the use of vegetable oils in the control of the citrus blackfly.

### Material and Methods

The experiments were carried out in the Entomology Laboratory, Crop Science Department, and in experimental field at the Agrarian Sciences Center (UFPB/Campus II) from April to June 2010. Treatments were oils of soybean (*Glycine max* (L.) Merrill, 1939), maize (*Zea mays* L., 1972), sunflower (*Helianthus annuus* L., 1939), cotton (*Gossypium hirsutum* L., 1910) and the commercial oil Rot Nim® in four concentrations [0 (control), 0.5, 1.0 and 1.5%]. The dilution of the oils was performed according to Medeiros *et al.* (2001). Dilutions were applied on the eggs and fourth-instar nymphs of *A. woglumi*.

**Source of vegetable oils and insects.** Egg and fourth-instar nymphs of *A. woglumi* were collected from orange plants

“Pear” (*Citrus sinensis* L. Osbeck) in Areia city - PB in commercial areas.

Vegetable oils of soybean, maize, and sunflower came from vegetal oil companies sold commercially and used in Brazilian cuisine, the cotton oil was provided by the Cotton National Research Center/Embrapa and Rot Nim® was purchased from a commercial provider. The description of the composition of vegetable oils is described in Table 1.

### Application of oils and evaluation of their insecticidal activity.

**Fourth-instar nymphal stage assay.** The oils mentioned above were tested in the following concentrations 0.0; 0.5; 1.0 and 1.5%, corresponding to 0; 5; 10 and 15 mL/L. In total 10 leaves/treatment were used, and each leaf was considered a repetition. Experiments were conducted on the same day in which the leaves were harvested. A laboratory experiment was conducted on the applications of oils through the immersion of citrus black fly infested leaves inside a 500 mL beaker per 5 seconds. The leaf sample was then placed on paper towels for five minutes to remove the excess solution. While the samples dried, a cotton ball was placed around the petioles of the treated leaves to avoid drying. Then, the samples were isolated in Petri dishes and stored in an environment chamber at 26 °C and 80% relative humidity. The evaluation of the insecticidal activity was done by counting the number of dead nymphs in cm<sup>2</sup> of each leaf, 15 days after the application of the oil treatments. The treatments were made in a 5 x 4 factorial scheme (5 treatments oil x 4 concentrations) consisting of 10 repetitions for each oil treatment. Insect mortality was evaluated by counting the number of dry insects in each cm<sup>2</sup> /leaf. The results of the mortality test were submitted to variance analysis, and the averages were compared by Tukey test ( $P \leq 0.05$ ), by using the program ASSISTAT 7.5 beta (ASSISTAT 2010).

**Egg stage assay.** The applications of vegetable oils were carried out in an experimental field, in the same concentrations as were used on pupae. An orange plant variety “Pear” (*C. sinensis*) intensely infested with adult *A. woglumi* was used. It was identified an egg laying/leaf before the treatment application in order to monitor its development after using the vegetable oils. The application of the oil on the insects was made through the immersion of infested leaves inside a 500 mL beaker for 5 seconds. The statistical analysis for this parameter was similar to the used for the pupal stage. The evaluation of the insecticidal activity on black fly eggs was

**Table 1.** Composition of vegetable oils in citrus black fly treatments.

Vegetable oil	Composition
Soybean	Soybean oil (genetically modified from <i>Agrobacterium</i> sp.); Citric acid antioxidant
Maize	Refined maize oil; citric acid antioxidant.
Sunflower	Refined sunflower oil; citric acid antioxidant (INS 330).
Cotton	Palmitic fat acid; oleic and linoleic; vitamin E (natural antioxidant).
Rot Nim®	<i>Azadirachta indica</i> (neem); <i>Derris</i> sp. (derris); <i>Chrysanthemum cinerariaefolium</i> (pyrethrum); <i>Piper nigrum</i> (black pepper); acetic acid (khaki vinegar); <i>Allium sativum</i> (garlic); <i>Allamanda nobilis</i> (yellow bell); <i>Mirabilis jalapa</i> (clavilia); <i>Melia azedarach</i> (chinaberry).

**Table 2.** Mortality of fourth instar nymphs of *Aleurocanthus woglumi* after 15 days following treatment with selected oils.

Oils	Concentrations (%)			
	0	0.5	1.0	1.5
Soybean	0.00 ± 0.00aC	100.00 ± 0.00aA	100.00 ± 0.00aA	100.00 ± 0.00aA
Maize	0.00 ± 0.00aD	100.00 ± 0.00aA	100.00 ± 0.00aA	100.00 ± 0.00aA
Sunflower	0.00 ± 0.00aD	100.00 ± 0.00aA	100.00 ± 0.00aA	100.00 ± 0.00aA
Cotton	0.00 ± 0.00aD	100.00 ± 0.00aA	100.00 ± 0.00aA	100.00 ± 0.00aA
Rot Nim®	0.00 ± 0.00aB	80.17 ± 19.83bB	100.00 ± 0.00aA	100.00 ± 0.00aA
CV (%)	7.29			

Averages ± standard deviation followed by capital letter, in row, and lower case letter, in column, for each variable, do not differ significantly by the Tukey test at level 5% of probability.

made based upon the percentage of non-viable eggs present in the demarcated egg count before the application of vegetable oils and 30 days after the application of the oils, it was considered as non-viable the eggs which were dark colored and shriveled. The mortality results of the eggs stage were submitted to variance analysis [Factorial (5 treatments oil x 4 concentrations)] and the averages were compared by Tukey test ( $P \leq 0.05$ ), through the program ASSISTAT 7.5 beta (UFCEG 2010).

### Results and Discussion

**Mortality of *A. woglumi* nymphs.** The effectiveness in the application of the selected vegetable oils on the mortality of citrus black fly nymphs was over 90% in doses over 1.0%. The highest mortality rates were achieved with soybean, maize, sunflower and cotton oils (100%) for all concentrations. On the other hand, Rot Nim® oil resulted in 80.2% mortality at 0.5% concentration which could be considered effective under an integrated pest management program (Table 2). Takatsuka and Czepak (2003) when treating leaves of the cotton plant cultivar ITA 90 with neem oil at a dosage of 1% observed a significant reduction of the population of living white aphids up to three days after the application; however, the Indian oil of nim in the dosage of 3% resulted in the control efficiency of the aphids over 80% up to six days after the application.

The citrus black fly feeds on its host through the insertion of its mouthparts in the leaf. Oils applied directly on the leaves provided a smooth surface on the leaves, which inhibit the fixation of the insects, prevents its feeding, and consequently

protects the plant from damages. The oils, which obstruct the spiracles of the insect, hinder gas exchange and provoke its death by asphyxia. The residues of the spray moves by capillarity motion to the aeropiles of the eggs, spiracles and tracheae of the larvae, nymphs and adults (De Ong *et al.* 1927; Johnson 1985). Moreover, the oils provoke alteration in the color and dehydration the insects, making them non-viable. The application of the oils, besides providing high population reduction of the citrus black fly, also causes a notable indirect action and visible reduction of sooty molds on leaves.

**Mortality of *A. woglumi* eggs.** The non-viability of eggs varied among the evaluated vegetable oils. Cotton oil provided 100% mortality in all concentrations, whereas sunflower oil at 0.5% resulted in only 20% of unviable eggs. Control treatment resulted in 0 % of embryonic mortality (Table 3).

According to the tests performed at Embrapa Meio-Norte (2009) with soybean, neem and castor bean oils in a concentration of 2%, it was observed that the oils used provided efficiency in the control of the black fly which varied from 45.9% to 70.7% and from 54.9% to 92.5% in the control of nymphs between the 2<sup>nd</sup> and the 20<sup>th</sup> day after application. Souza and Vendramim (2000) provided evidence on the lethal effect on eggs of the whitefly *Bemisia tabaci* (Gennadius), when treated with leaf watery extract of *Melia azedarach* (L., 1854) and branches of *Trichilia pallida* (Swartz, 1849), in variable concentrations of 1.0 - 3.0%; the highest mortality value was obtained for *T. pallida* at a concentration of 3% (52.3%). In this study, the lowest concentration of vegetable oils (0.5%) affected the viability eggs citrus black fly (up to 90%), suggesting its suitability for integrated pest management programs.

**Table 3.** Mortality of eggs of *Aleurocanthus woglumi* after treatment with vegetable oils.

Oils	Concentrations (%)			
	0	0.5	1.0	1.5
Soybean	0.00 ± 0.00eA	100.00 ± 0.00aA	100.00 ± 0.00aA	25.53 ± 21.80cB
Maize	0.00 ± 0.00eA	61.90 ± 0.00bA	48.28 ± 0.00bB	36.58 ± 0.00bC
Sunflower	0.00 ± 0.00eA	20.00 ± 0.00dC	79.41 ± 22.73bB	100.00 ± 0.00aA
Cotton	0.00 ± 0.00eA	100.00 ± 0.00aA	100.00 ± 0.00aA	100.00 ± 0.00aA
Rot Nim®	0.00 ± 0.00eA	40.00 ± 0.00dC	66.07 ± 0.00cB	100.00 ± 0.00aA
C.V.* (%)	7.29			

Averages ± standard deviation followed by capital letter, in row, and lower case letter, in column, for each variable, do not differ significantly by the Tukey test at level 5% of probability; C.V. - Coefficient of variation.

**Table 4.** Viability of eggs of *Aleurocanthus woglumi* after treatment with vegetable oils.

Oils	Concentrations (%)			
	0	0.5	1.0	1.5
Soybean	50.76 ± 16.64aA	26.98 ± 16.72aBC	33.56 ± 13.49aB	16.34 ± 6.43bC
Maize	50.76 ± 16.64aA	15.25 ± 11.13abC	13.97 ± 2.02bC	31.05 ± 9.34abB
Sunflower	50.76 ± 16.64aA	12.11 ± 4.37bB	17.15 ± 5.76bB	43.88 ± 16.13aA
Cotton	50.76 ± 16.64aA	0.00 ± 4.22dA	0.00 ± 6.99cA	0.00 ± 8.36bA
Rot Nim®	50.76 ± 16.64aA	26.20 ± 18.51abB	40.00 ± 0.00aAB	27.47 ± 3.68bB
C.V.* %	39.08			

Averages ± standard deviation followed by capital letter, in row, and lower case letter, in column, for each variable, do not differ significantly by the Tukey test at level 5% of probability; C.V. - Coefficient of variation.

For the egg hatching index (viability of eggs), Rot Nim® at 1.0% provided the highest egg hatching percentage (40%); cotton oil caused the lowest percentage of egg hatching (0%) (Table 4).

Some of the insects in the experiment completed the embryonic development, but died without being able to break completely the egg chorion. This suggests that, in these cases, the oils would not have affected embryogenesis. This type of effect also was observed by Liu and Stansly (1995) and by Prabhaker *et al.* (1999), after the application of commercial oil and the insecticide amitraz, respectively, on eggs of *B. tabaci* biotype B. According to Prabhaker *et al.* (1999), this would possibly have occurred due to the contact of the insect with the residues of the extracts on the chorion.

After the use of vegetable oils, the eggs displayed a dark coloration and the nymphs which hatched became desiccated and consequently died. This indicates that these vegetable oils possibly make the embryos unviable (so the eggs do not hatch). In the concentrations used, there was no incidence of phytotoxicity to the plant.

### Conclusions

The efficiency in the mortality of pupae of *A. woglumi* was observed for each of the evaluated plant oils in the highest concentrations reaching 100% of mortality. The cotton oil provided greater ovicidal action and lower percentage of egg hatching, indicating to be a promising product in an integrated pest management program of *A. woglumi*.

The most lethal concentrations of vegetable oils for the nymphal stage of *A. woglumi* were at 1.0 and 1.5% and for eggs the most lethal concentration was at 1.0%.

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