Insecticidal and Behavioral Effects of Secondary Metabolites from Meliaceae On Bemisia tabaci (Hemiptera: Aleyrodidae)

Authors: Bezerra-Silva, Gerane Celly Dias, Silva, Márcio Alves, Vendramim, José Djair, and Dias, Carlos Tadeu Dos Santos

Source: Florida Entomologist, 95(3) : 743-751

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.095.0325

INSECTICIDAL AND BEHAVIORAL EFFECTS OF SECONDARY METABOLITES FROM MELIACEAE ON *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE)

Gerane Celly Dias Bezerra-Silva[1,*], Márcio Alves Silva[1], José Djair Vendramim[1], and Carlos Tadeu Dos Santos Dias[2]
[1]Department of Entomology and Acarology and [2]Department of Exact Sciences; University of São Paulo, ESALQ, CP 9, CEP 13418-900, Piracicaba, SP, Brazil

*Corresponding author; E-mail: gcdbezerra@gmail.com

Abstract

We studied the effects of crude extracts and fractions of *Azadirachta indica*, *Melia azedarach*, *Toona ciliata* and *Trichilia pallida* on both egg and nymph mortality and embryonic development of *Bemisia tabaci* B biotype, using tomato plants grown in a greenhouse. Next, we studied the host selection behavioral effects on the adult whitefly under laboratory conditions. The dichloromethane extracts from all plant species and fractions of the extract from branches of *T. pallida* (EBTPD) and of the extract from leaves of *T. ciliata* (ELTCD) in dichloromethane caused mortality of nymphs, but neither affected egg viability. However, the branches of the ethanolic extract of *A. indica* increased the period of embryonic development of the *B. tabaci*. In addition, the tomato leaflets treated with the fraction of ELTCD dichloromethane (0.28%) were the least preferred by adults, reducing the number of insects resting on the tomato leaflets. The ELTCD methanol and EBTPD dichloromethane fractions inhibited *B. tabaci* oviposition. Thus, Meliaceae derivatives can contribute to the reduction of the *B. tabaci* population. The susceptibility of the *B. tabaci* to Meliaceae derivatives and the relevant behavioral changes of this pest are discussed.

Key Words: whitefly, *Azadirachta indica*, *Melia azedarach*, *Trichilia pallida*, *Toona ciliata*

Resumo

Avaliou-se o efeito de extratos brutos e frações de *Azadirachta indica*, *Melia azedarach*, *Toona ciliata* e *Trichilia pallida* sobre a mortalidade ovos e ninfas e desenvolvimento embrionário da mosca-branca *Bemisia tabaci* biótipo B em plantas de tomate em casa de vegetação. Além disso, os efeitos sobre o comportamento de seleção hospedeira dos adultos foram registrados. Os extratos brutos de todas as espécies vegetais e frações do extrato diclorometânico de ramos de *T. pallida* e do extrato de folhas de *T. ciliata* ocasionaram mortalidade de ninfas, mas não afetaram a viabilidade dos ovos. Por outro lado, o extrato etanólico de ramos de *A. indica* aumentou o período embrionário da mosca-branca. Adicionalmente, os folíolos pulverizados com a fração em diclorometano do extrato diclorometânico de folhas de *T. ciliata* foram menos preferidos pelos adultos. As frações em diclorometano e em metanol dos extratos diclorometânicos de ramos de *T. pallida* e de folhas de *T. ciliata*, respectivamente, inibiram a oviposição da mosca-branca. Assim, o uso de extratos de Meliaceae contribui para reduzir a população de *B.tabaci* biótipo B. A susceptibilidade da mosca-branca para derivados de Meliaceae e suas mudanças de comportamento são discutidos.

Palavras Chave: mosca-branca; *Azadirachta indica*; *Melia azedarach*; *Trichilia pallida*; *Toona ciliata*

Secondary metabolites are mediators of interactions between plants and other organisms. The Sapindales are considered to be one of the richer and diverse sources of secondary metabolites in angiosperms (Waterman 1993). Within a broad spectrum of compound classes found in Sapindales, limonoids stand out (Fang et al. 2011). In particular, these compounds characterize members of the family Meliaceae as they are diverse and abundant (Champagne et al. 1992). More than 130 limonoids already were isolated from different parts of the neem tree, *Azadirachta indica* A. Juss (Sapindales: Meliaceae: Melioideae) (Kanokmedhakul et al. 2005). The azadirachtin is the most investigated and is considered to possess the most potential to be used in the integrated pest management (IPM) programs (Morgan 2009). The identification of limonoids and their subsequent synthesis for IPM has been the subject of research (Heasley 2011). However, the use of a single synthetic limonoid for the IPM of whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is not convenient and would result in the same mistakes experienced with other synthetic insecticides. The genetic variability of the *B. tabaci*, which is a complex of 11 well-defined high-level groups containing at least 24 morphologically indistinguishable species (De Barro et al. 2011), and changes in agricultural systems have contributed to these species becoming a major pest in modern agricultural practice. The use of pure compounds such as azadirachtin increases the likelihood of developing resistant insect populations (Feng & Isman 1995). However, Meliaceae derivatives often contain a mixture of active substances, which can delay or prevent the development of resistance.

Due to the positive results experienced with neem, other Meliaceae extracts are being investigated with the intent of their application in IPM programs, especially in organic agriculture. In this context, we evaluated the effect of organic extracts from trees belonging to the subfamily Melioideae (*A. indica*, *Melia azedarach* L. and *Trichilia pallida* Swartz) and to the subfamily Swietenioideae (*Toona ciliata* M. Roemer) on eggs and nymphs of *B. tabaci* B biotype maintained on tomato plants grown in a greenhouse. Then, to maximize the biological activity and reproducibility of the action of the derivatives, we fractionated the branch extract of *T. pallida* and the leaf extract of *T. ciliata* in dichloromethane, and evaluated their effects on nymphs of the whitefly on tomato plants in a greenhouse. We also evaluated the effects of these fractions on host selection of *B. tabaci* adults under laboratory conditions.

The subfamily Melioideae is characterized by the presence of limonoids with an intact carbon skeleton (Champagne et al. 1992). In this subfamily, the genera *Azadirachta* and *Melia* (Melioideae: Meliae) possess similar compounds, such as the highly oxidized C-seco limonoids. However, only the first genus contains species that produce the limonod, azadirachtin (Morgan 2009). Additionally, the genus *Trichilia* (Melioideae: Trichilieae) has the highest number and highest variability of limonoids, while the trees of the genus *Toona* (Swietenioideae: Cedreleae) are similar to the Melioideae in that they have limonoids with an intact carbon skeleton (Oiano-Neto et al. 1995).

Materials and Methods

Insects and Tomato Plants

Bemisia tabaci adults were acquired from colonies maintained at the entomology sector of the Agronomic Institute of Campinas (IAC), Campinas, São Paulo State, Brazil, previously identified as *B. tabaci* B-biotype by induction of silvering in pumpkin leaves. The *B. tabaci* colony was reared in a greenhouse (approximately 2.5 m^2) with anti-aphid screens and without automatic environmental controls. Soybean plants [*Glycine max* (L.) Merrill, cv. IAC-24] were grown in 3L plastic bags and used as hosts for insect rearing. New plants were introduced every 15 d to replace old plants already weakened by the high whitefly population. For the experiments, seeds of tomato plants of cv. 'Santa Clara' were planted in plastic trays containing Plantmax Hortaliças HT® substrate (DDL®Agroindústria, Betel Paulínia, SP, Brazil). Fifteen d after sowing, the seedlings were transplanted into 0.5 L plastic bags containing the same substrate for germination. The tomato plants used in this study were 30 d old.

Plant Materials for Preparing Meliaceae Extracts

Leaves and branches were collected from *A. indica*, *M. azedarach*, *T. ciliata* and *T. pallida* trees located at the "Luiz de Queiroz" College of Agriculture campus in Piracicaba (S 22° 42' W 47° 37'), São Paulo State, Brazil. These species were selected and tests were conducted with organic extracts (non-aqueous) of their tissues, because promising insecticidal activity had been found with aqueous extracts of these plant materials.

Preparation of Crude Extracts and Different Solvent Fractions of Meliaceae Structures

The leaves and branches were dehydrated in an oven at 40 °C for 48 to 96 h, then ground into powders in a knife mill, and the resulting powders were kept in hermetically closed glass flasks. Two organic extracts from each part of the plant (leaves and branches) were obtained with the solvents dichloromethane and ethanol. To obtain the extracts, the plant powders were subjected to a Soxhlet extraction system. A sample of 40 g of powder of each structure was placed into a five filter paper cartridge Soxhlet extractor along with 300 mL of solvent for leaf and seed powders, and with 200 mL for branch powders. The extraction time varied according to the structure used. The leaf and branch samples were kept under reflux during 16 h and 10 h, respectively. The extraction endpoint was set by the color change of the solvent, i.e., when the solvent passed through the sample and remained with no color, indicating that the extraction had reached its maximum. This procedure was applied with each of the solvents and each plant powder, and repeated until completion of extraction of the total mass. Then, the extracts were concentrated using a swivel evaporator at 40 °C, low pressure, connected to a water hose. After this process, the extracts were stored in glass flasks and kept in a laminar flow cabinet to allow the complete evaporation of the solvents (Roel et al. 2000a,b; Cunha et al. 2005; 2006).

To obtain the fractions, the extracts of branches of *T. pallida* (EBTPD) and leaves of *T. ciliata*

(ELTCD) were fractionated using vacuum liquid chromatography (CLV), and a sintered funnel plate in two phases: a stationary phase (common silica, 230-400 mesh) and a mobile phase (4 solvents with increasing polarity), resulting in hexane, dichloromethane, ethyl acetate and methanol fractions. These fractions were concentrated in a rotary evaporator at 40 °C at a low pressure using water hoses, and these fractions were later placed in glass flasks in a laminar flow cabinet until the solvents evaporated completely.

Effects of Meliaceae Crude Extracts on the Survival of *Bemisia tabaci* Eggs

To obtain *B. tabaci* eggs we used 15 cm diam cylindrical sleeve cage covered with voile, which could be placed over a tomato leaf and opened and closed by a string at either end. One cage was installed on each tomato plant. Forty unsexed adults of the whitefly were released into each cage, and maintained for 24 h for oviposition; and then the adults were removed. The tomato plants were taken to the laboratory, and the number of eggs on the abaxial surface of the apical leaflet was counted. Glass tubes (9.0 × 2.5 cm) were covered with Parafilm® with a hole in the center for insertion of a straw. The apical leaflet with 30 eggs was cut from each plant and inserted through the straw. Each glass tube was filled with deionized water to maintain the turgidity of the leaflets. Then, the leaflets were sprayed with one of the following extracts: (i) branches of *T. pallida* in dichloromethane (EBTPD), (ii) leaves of *A. indica* in ethanol (ELAIE), (iii) branches of *A. indica* in ethanol (EBAIE), (iv) branches of *M. azedarach* in ethanol (EBMAE), (v) leaves of *T. ciliata* in dichloromethane (ELTCD), and (vi) branches of *T. ciliata* in dichloromethane (EBTCD). Each of these sprays had a concentration of 0.56%, based a findings of Bezerra-Silva et al. (2010). Also two controls, deionized water and acetone, were used because the extracts have been dissolved in these solvents. Beginning on the sixth day, the number of nymphs and non-viable eggs was counted.

We used these data to calculate the mortality at the egg stage and the duration of the embryonic development. The bioassay was conducted using a randomized design with 8 treatments and 7 replicates, and each repetition corresponded to a leaflet. According with the previous results obtained by Bezerra-Silva et al. (2010), 3 extracts in dichloromethane and 3 in ethanol that caused elevated mortality of nymphs, were selected for comparison in this study and from this comparison the two most efficient were selected for fractionation.

Effects of Meliaceae Crude Extracts on the Survival of *Bemisia tabaci* Nymphs

The procedure to infest tomato leaves with *Bemisia tabaci* adults for obtaining eggs to develop into the nymphs to be tested was the same as in the previous experiment. Nine days after infestation when the nymphs were with 2-3 d old and feeding on the plant, the effects of the extracts on *B. tabaci* nymph survival was assessed. Thereafter, the leaflets were sprayed with the extracts (the same utilized for eggs) at a concentration of 0.56%, and controls were treated with either deionized water or acetone. The plants were kept in a greenhouse, and 7 d after spraying, we counted the number of dead nymphs. The bioassay was conducted in a randomized design with 6 treatments and 4 replicates, and each repetition corresponded to 2 leaflets per tomato plant. At the end of this stage, we selected dichloromethane extracts of *T. pallida* branches (EBTPD) and of *T. ciliata* leaves (ELTCD) to perform the fractionation steps, because we found that these extracts have caused elevated mortality of nymphs.

Effects of Different Fractions of Meliaceae Crude Extracts on Survival of Nymphs

We counted the nymphs on each leaflet 9 d after infestation, when the nymphs were 2-3 d old and feeding on the plant. The EBTPD (dichloromethane, ethyl acetate and methanol) and ELTCD (hexane, dichloromethane, ethyl acetate and methanol) fractions were sprayed on the leaflets. Each fraction was diluted with a mix of acetone and deionized water (proportion 1:4) to create concentrations of 0.28%. Deionized water alone and a mix of acetone and deionized water (proportion 1:4) were used as controls. The plants were kept in a greenhouse, and 7 d after spraying, the numbers of dead nymphs were counted. The bioassay was conducted in a randomized design with 11 treatments involving 7 fractions, 2 extracts (EBTPD and ELTCD) and 2 controls and 4 repetitions consisting of 2 leaflets per tomato plant. Later, we began a new test using the same procedures previously described. However, this time the fractions were prepared at a concentration of 0.56% and were diluted with pure acetone. Bioassays were conducted in a randomized design with 9 treatments involving 7 fractions and 2 controls (deionized water and pure acetone) and were performed with 7 repetitions consisting of 2 leaflets per tomato plant.

Effects of Different Fractions of Meliaceae Crude Extracts on Host Selection Behavior of *B. tabaci*

To assess the potential host selection behavioral effects caused by the different fractions, we used plastic cages each 16 cm high × 13 cm in diam. In the lids of these containers, a 6 cm diam opening covered with anti-aphid netting allowed for aeration. Inside the containers, 2 plastic holders (5 cm long) filled with deionized water in plastic holders were attached along the inner wall on opposite sides of the cages. On one side of the cage we placed a leaflet treated with the fraction and on the other side, a leaflet treated with deionized water. The leaflets were sprayed either with the fractions at a concentration of 0.28% or with deionized water, and they were then left on filter paper for approximately 10 min to remove excess moisture. A lateral hole allowed the introduction of insects. Each cage, containing two tomato leaflets, was infested with 20 pairs of whiteflies for 24 h. After this period, we recorded the number of adults and eggs on the abaxial surface of each leaflet. This experimental design was completely randomized with 7 treatments (fractions) and 8 repetitions.

Statistical Analysis

Data from tests of mortality of eggs and nymphs and on embryonic development were analyzed by a one-way analysis of variance F-test. If a significant difference was detected, Tukey's honestly significant difference (HSD) test was used to compare the means. The Bartlett (Bartlett 1937) and Shapiro-Wilk (Shapiro & Wilk 1965) tests were used to evaluate homoscedasticity and the normality of the residuals, respectively. Without these assumptions, the data were transformed by the Method of Box-Cox Optimal Power (Box & Cox 1964). Analyses were performed using the SAS 9.1 statistical program (PROC GLM, SAS Institute 2003). Additionally, we determined the efficiency of extracts using Abbott's formula (1925) to adjust the data on mortality of the eggs and nymphs taking into account the highest value of the mortalities observed in the controls (deionized water or acetone). To study the effects of treatments on host selection behavior, the inhibition index (II) adapted from Kogan & Goeden(1970) by Silva et al. (2011) was applied. This index is calculated using the formula II = 2G/(G+P), where G is the percent of eggs or adults on the treated tomato leaflet, and P is the percent of eggs or adults on the control tomato leaflet. Based on the II and on the standard deviations obtained, the classification intervals (CI) for the means of the treatments were estimated by the formula

$$\mathrm{CI} = 1 \pm t_{(n-1;\,\alpha = 0.05)} \times \frac{SD}{\sqrt{n}}$$

where t is the value of Student's t distribution ($P < 0.05$), SD is the standard deviation, and n is the number of replicates. The fraction was considered to have no effect if the estimated II value was within the CI range. The fraction was considered to have an inhibitory effect if the II value was less than the lower bound of the CI. The fraction was considered to have a stimulating effect if the II value was greater than the upper bound of the CI.

Results

Effects of Meliaceae Crude Extracts on the Survival of Eggs and Nymphs

The period of embryonic development of the whitefly was increased by the *A. indica* branches ethanol extract, because the treated embryos had a duration of 8.0 d ($F = 28.98$; df = 2.10; $P < 0.0001$) compared to all of the other extract treatments and controls in which this period varied between 7.0 and 7.2 d, but none of these differed from each other statistically (Table 1). With regard to embryonic mortality, there were differences between treatments ($F = 5.13$; df = 21.61; $P = 0.0002$); however, the activity of extracts did not differ from the deionized water control (Table 1). Each of the Meliaceae extracts caused a high mortality rate of the *B. tabaci* nymphs ($F = 27.05$;

Table 1. Mortality and embryonic development (± SE) of *Bemisia tabaci* B biotype eggs on tomato after application of Meliaceae extracts (0.56%). (LD 14: 10 H; 25 ± 2 °C; 66 ± 10% relative humidity).

Treatments	Eggs / leaflet	Embryonic development (days)*	[1]Mortality (%)*	[2]Efficiency (%)
Deionized water	38.7 ± 5.1	7.1 ± 0.06 a	8.3 ± 5.8 abc	—
Acetone	33.0 ± 3.5	7.1 ± 0.03 a	1.4 ± 1.9 c	—
EBTPD[3]	33.0 ± 4.7	7.2 ± 0.05 a	33.5 ± 21.0 a	27.4
ELAIE[4]	75.1 ± 18.9	7.1 ± 0.10 a	13.2 ± 8.9 abc	5.4
EBAIE[5]	33.1 ± 8.7	8.0 ± 0.04 b	18.5 ± 18.8 ab	11.1
EBMAE[6]	30.1 ± 5.4	7.1 ± 0.06 a	9.2 ± 8.4 abc	1.0
ELTCD[7]	34.6 ± 6.9	7.1 ± 0.06 a	4.9 ± 5.0 bc	0.0
EBTCD[8]	56.3 ± 7.1	7.0 ± 0.02 a	6.3 ± 20.4 a	19.6

*Means followed by different letters in the columns differ by Tukey's test ($P < 0.05$).
[1]Original means in this table. For analysis, the data were transformed using $\lambda = (x + 0.5)^{(0.2)}$.
[2]Determined after correcting the control mortality by Abbott's formula (1925).
[3]EBTPD: Extract of branches of *Trichilia pallida* in dichloromethane;
[4]ELAIE: Extract of leaf of *Azadirachta indica* in ethanol;
[5]EBAIE: Extract of branches of *Azadirachta indica* in ethanol;
[6]EBMAE: Extract of branches of *Melia azedarach* in ethanol;
[7]ELTCD: Extract of leaf of *Toona ciliata* in dichloromethane;
[8]EBTCD: Extract of branches of *Toona ciliata* in dichloromethane.

df = 14.45; $P < 0.0001$) (Table 2). All of extract mortalities differed from the controls, but there was no difference between the extracts, regardless of the species of Meliaceae, plant structure, or solvent that was used (Table 2).

Effects of Different Fractions of Meliaceae Crude Extracts on Survival of Nymphs

With the exception of the ELTCD ethyl acetate fraction (*T. ciliata* leaf), all fractions of the EBTPD (*T. pallida* branch) and ELTCD at 0.28% caused significantly higher mortalities of whitefly nymphs than the controls ($F = 16.10$; df = 19.87; $P< 0.0001$) (Table 3). Also all fractions at 0.56% caused significantly higher mortalities of whitefly nymphs (75.6 to 97.4%) than the controls ($F = 58.64$; df = 16.25; $P < 0.0001$) (Table 4). The greatest mortality rate (96.9% efficiency) was caused by the *T. ciliata* leaf fraction, ELTCD ethyl acetate, which differed significantly from the mortality rates of both the *T. pallida* branch fraction, EBTPD ethyl acetate, and the *T. ciliata* leaf fraction, ELTCD dichloromethane. The mortalities caused by the other treatments were not significantly different from either the above mentioned treatments with the highest mortalities or those with the lowest mortalities (Table 4).

Effects of Different Fractions of Meliaceae Crude Extracts on Host Selection Behavior of *B. tabaci*

The number of *B. tabaci* adults on tomato leaflets after 24 h of exposure was lowest in the dichloromethane fraction of ELTCD (Table 5). In this case, approximately 70% of the insects released in the cage were found on the leaflets (treated + control), of which, 81.9% were observed on the control leaflets and 18.1% on the treated leaflets (Table 5). This fraction caused an inhibitory effect on landing and/or staying on the tomato leaflet (II = 0.36 ± 0.41) (Table 5). Additionally, ovipositional behavior of the *B. tabaci* on tomato leaflets was inhibited by fractions of EBTPD dichloromethane (II = 0.14 ± 0.09) and ELTCD methanol (II = 0.31 ± 0.12) (Table 6). The opposite effect was observed with the fraction of EBTPD methanol, indicating that this behavior is an oviposition stimulant (II = 1.32 ± 0.11) (Table 6). The remaining fractions had a neutral impact on settling and oviposition on tomato leaves by *B. tabaci* (Tables 5 and 6).

Discussion

Plant derivatives of several Meliaceae species have low ovicidal activity on *B. tabaci* eggs (Prabhaker et al. 1989; Price et al. 1990), especially the B biotype (Souza &Vendramim 2000a,b). Here, the organic extracts from Meliaceae that were studied showed no significant ovicidal activity (Table 1). The egg chorion is waterproof, so even if a Meliaceae extract reaches the eggs, it may have difficulty penetrating this barrier and causing harmful effects. The observed mortality was caused by the inability of fully developed nymphs to break the egg chorion or to detach completely (Prabhaker et al. 1989). Death at this time probably was caused by contact with residue on the egg surface (see also Prabhaker et al. 1989; Liu & Stansly 1995; Prabhaker et al. 1999). However, the compounds present in the branches of ethanol extract of *A. indica* prolonged the embryonic development of *B. tabaci*. The increase in the embryonic development period may be caused by the presence of azadirachtin in the extract. Azadirachtin prolonged the embryonic development and reduced the hatchability of various insects (Mordue [Luntz] et al. 2005; Ghazawy et al. 2010). However, this low ovicidal activity is offset by the much greater effectiveness of azadirachtin

Table 2. Mortality (±SE) of *Bemisia tabaci* B biotype nymphs on tomato after application of Meliaceae extracts (0.56%). (LD 14: 10 h; 25 ± 2 °C; 66 ± 10% relative humidity).

Treatments	Nymphs / leaflet	Mortality (%)[1,*]	Efficiency (%)[2]
Deionized water	161.3 ± 16.0	2.0 ± 0.3 c	—
Acetone	115.0 ± 30.6	32.7 ± 10.1 b	—
EBTPD[3]	120.5 ± 37.2	93.9 ± 2.1 a	90.9
ELAIE[4]	179.5 ± 26.6	89.5 ± 4.7 a	84.4
EBAIE[5]	114.5 ± 35.3	80.5 ± 8.4 a	71.0
EBMAE[6]	119.8 ± 26.5	86.0 ± 9.2 a	79.2
ELTCD[7]	109.8 ± 24.0	93.5 ± 2.0 a	90.4
EBTCD[8]	93.3 ± 24.2	71.8 ± 16.3 a	58.1

[*]Means followed by different letters in the columns differ by Tukey's test ($P < 0.05$).
[1]Original means in this table. For analysis, the data were transformed using $\lambda = \sqrt{x}$.
[2]Determined after correcting the control mortality by Abbott's formula (1925).
[3]EBTPD: Extract of branches of *Trichilia pallida* in dichloromethane;
[4]ELAIE: Extract of leaf of *Azadirachta indica* in ethanol;
[5]EBAIE: Extract of branches of *Azadirachta indica* in ethanol;
[6]EBMAE: Extract of branches of *Melia azedarach* in ethanol;
[7]ELTCD: Extract of leaf of *Toona ciliata* in dichloromethane;
[8]EBTCD: Extract of branches of *Toona ciliata* in dichloromethane.

TABLE 3. MORTALITY (±SE) OF *BEMISIA TABACI* B BIOTYPE NYMPHS ON TOMATO AFTER APPLICATION OF DIFFERENT FRACTIONS OF MELIACEAE EXTRACTS (0.28%). (LD 14: 10 H; 25 ± 2 °C; 65 ± 10% RELATIVE HUMIDITY).

Treatments	Nymphs / leaflet	Mortality (%)[1,*]	Efficiency (%)[2]
Deionized water	156.3 ± 24.7	13.6 ± 7.4 c	—
Mix (acetone: deionized water)	105.5 ± 10.5	12.7 ± 6.4 c	—
Fraction of EBTPD dichloromethane[3]	112.0 ± 24.0	54.4 ± 13.3 ab	47.2
Fraction of EBTPD ethyl acetate[3]	156.0 ± 24.8	65.1 ± 5.0 a	59.6
Fraction of EBTPD methanol[3]	98.5 ± 18.5	65.6 ± 3.8 a	60.2
Fraction of ELTCD hexane[4]	115.5 ± 13.9	50.6 ± 7.1 ab	42.9
Fraction of ELTCD dichloromethane[4]	125.8 ± 15.4	50.7 ± 8.4 ab	43.0
Fraction of ELTCD ethyl acetate[4]	130.0 ± 9.9	36.7 ± 7.2 bc	26.7
Fraction of ELTCD methanol[4]	125.5 ± 8.3	75.2 ± 3.4 a	71.3
EBTPD[3]	145.8 ± 27.1	73.6 ± 3.0 a	69.4
ELTCD[4]	131.5 ± 25.4	65.7 ± 3.1 a	60.3

[*]Means followed by different letters in the columns differ by Tukey's test ($p < 0.05$).
[1]Original means in this table. For analysis, the data were transformed using $\lambda = \sqrt{x}$.
[2]Determined after correcting the control mortality by Abbott's formula (1925).
[3]EBTPD: Extract of branches of *Trichilia pallida* in dichloromethane;
[4]ELTCD: Extract of leaf of *Toona ciliata* in dichloromethane;

and other limonoids by ingestion than by contact (Morgan 2009).

EBTPD and ELTCD showed an approximate 90% efficiency in controlling nymphs, which are the most susceptible of the stages of *B. tabaci* development; therefore, both crude extracts were selected for fractionation. The polarity of the solvent that was used for extraction or fractionation and biological activity are directly related (see Roel &Vendramim 1999; Roel et al. 2000a,b; Cunha et al. 2005; 2006). Here, the fractions with different solvents polarities had similar biological activities, indicating that the 2 species of Meliaceae, *T. pallida* and *T. ciliata* possess a high diversity of secondary metabolites with insecticidal activity on *B. tabaci*. The branches and leaves of these plants contain active compounds belonging to different chemical groups with similar insecticidal activity, and the fractionation of the extracts using solvents with increasing polarity did not reduce the control efficiency of the extracted material (i.e., the fraction). These results support the use of Meliaceae extracts in integrated management of *B. tabaci* because the greater diversity of the chemical groups with insecticidal activity in the extract lowers the likelihood for the development of resistant insect populations.

Management of *B. tabaci* populations is a difficult challenge because population reduction must be sufficiently stringent to essentially prevent the transmission of geminiviruses. The ideal plan would be to prevent *B. tabaci* inoculating virus during the phenological stage in which culture is more susceptible. The fraction of the ELTCD dichloromethane (0.28%), in addition to causing 70% mortality of nymphs, is also able to substantially inhibit the landing of the *B. tabaci* adults on the leaves. The EBTPD dichloromethane and ELTCD methanol fractions also reduce colonization of tomato plants by *B. tabaci*. These compounds inhib-

TABLE 4. MORTALITY (±SE) OF *BEMISIA TABACI* B BIOTYPE NYMPHS ON TOMATO AFTER APPLICATION OF DIFFERENT FRACTIONS OF MELIACEAE EXTRACTS (0.56%). (LD 14:10 H; 25 ± 2 °C; 65 ± 10% RELATIVE HUMIDITY).

treatments	Nymphs/leaflet	Mortality (%)[*]	Efficiency (%)[1]
Deionized water	55.3 ± 4.9	10.9 ± 9.5 c	—
Acetone	67.4 ± 8.8	17.1 ± 7.7 c	—
Fraction of EBTPD dichloromethane[3]	58.0 ± 3.9	94.8 ± 6.6 ab	93.7
Fraction of EBTPD ethyl acetate[3]	47.1 ± 4.2	75.6 ± 16.8 b	70.6
Fraction of EBTPD methanol[3]	53.6 ± 11.1	91.3 ± 8.8 ab	89.6
Fraction of ELTCD hexane[4]	62.4 ± 8.6	91.2 ± 10.4 ab	89.4
Fraction of ELTCD dichloromethane[4]	57.3 ± 8.1	76.3 ± 22.9 b	71.5
Fraction of ELTCD ethyl acetate[4]	58.4 ± 2.7	97.4 ± 5.3 a	96.9
Fraction of ELTCD methanol[4]	51.9 ± 6.5	94.8 ± 6.6 ab	93.7

[*]Means followed by different letters in the columns differ by Tukey's test ($p < 0.05$).
[1]Determined after correcting the control mortality by Abbott's formula (1925).
[3]EBTPD: Extract of branches of *Trichilia pallida* in dichloromethane.
[4]ELTCD: Extract of leaf of *Toona ciliata* in dichloromethane.

TABLE 5. INHIBITION OF *BEMISIA TABACI* B BIOTYPE ADULTS FROM SETTLING ON TOMATO LEAVES BY DIFFERENT FRACTIONS OF MELIACEAE EXTRACTS (0.28%) (LD 14:10 H; 25 ± 2 °C; 70 ± 10% RELATIVE HUMIDITY). DATA SHOW THE PERCENTAGES OF ADULTS THAT SETTLED ON TREATED LEAVES VS THOSE ON CONTROL LEAVES.

treatments	Adults (%)	II (M ± SE)[3]	CI[4]	[5]Classification
Fraction of EBTPD dichloromethane[1]	33.22	0.66 ± 0.61	(0.50; 1.50)	Null
Control	66.78			
Fraction of EBTPD ethyl acetate[1]	39.11	0.78 ± 0.38	(0.69; 1.31)	Null
Control	60.89			
Fraction of EBTPD methanol[1]	55.15	1.10 ± 0.39	(0.68; 1.32)	Null
Control	44.85			
Fraction of ELTCD hexane[2]	57.30	1.15 ± 0.45	(0.59; 1.41)	Null
Control	42.70			
Fraction of ELTCD dichloromethane[2]	18.13	0.36 ± 0.41	(0.63; 1.37)	Inhibitor
Control	81.87			
Fraction of ELTCD ethyl acetate[2]	24.97	0.50 ± 0.62	(0.44; 1.56)	Null
Control	75.03			
Fraction of ELTCD methanol[2]	46.67	0.93 ± 0.69	(0.37; 1.63)	Null
Control	53.33			

[1]EBTPD: Extract of branches of *Trichilia pallida* in dichloromethane.
[2]ELTCD: Extract of leaf of *Toona ciliata* in dichloromethane.
[3]II = Inhibition Index.
[4]CI=Classification Interval.
[5]Classification = Null: Included in the classification interval (CI < II < CI); Inhibitor: II <CI; Stimulant: II > CI.

ited the oviposition of *B. tabaci* in the culture. The decrease of the insect preference for plants with Meliaceae extracts can reduce the possibility of virus inoculation. The decreased preference of *B. tabaci* for plants treated with limonoids that are present in the derivatives of *A. indica* and *M. azedarach* has been well documented (Coudriet et al. 1985; Cubillo et al. 1994; Prabhaker et al. 1999; Abou-Fakhr Hammad et al. 2000; Abou-Fakhr Hammad et al. 2001; Kumar et al. 2005; Abou-Fakhr Hammad & McAuslan 2006; Baldin et al. 2007). In general, the following 4 mechanisms are involved in the inhibition of the host selection by allelochemicals: repellent effects, locomotor stim-

TABLE 6. INHIBITION OF OVIPOSITION OF *BEMISIA TABACI* B BIOTYPE BY DIFFERENT FRACTIONS OF MELIACEAE EXTRACTS (0.28%). (LD 14:10 H; 25 ± 2 °C; 70 ± 10% RELATIVE HUMIDITY).

treatments	Eggs / leaflet	II (M ± SE)[3]	CI[4]	Classification[5]
Fraction of EBTPD dichloromethane[1]	2.13	0.14 ± 0.09	(0.78; 1.22)	Inhibitor
Control	30.63			
Fraction of EBTPD ethyl acetate[1]	18.75	0.86 ± 0.17	(0.56; 1.44)	Null
Control	33.75			
Fraction of EBTPD methanol[1]	34.88	1.32 ± 0.11	(0.72; 1.28)	Stimulant
Control	16.00			
Fraction of ELTCDhexane[2]	16.88	0.81 ± 0.18	(0.55; 1.45)	Null
Control	25.63			
Fraction of ELTCD dichloromethane[2]	4.75	0.63 ± 0.21	(0.45; 1.55)	Null
Control	10.75			
Fraction of ELTCDethyl acetate[2]	10.88	0.68 ± 0.18	(0.54; 1.46)	Null
Control	19.25			
Fraction of ELTCD methanol[2]	3.88	0.31 ± 0.12	(0.68; 1.32)	Inhibitor
Control	25.13			

[1]EBTPD: Extract of branches of *Trichilia pallida* in dichloromethane.
[2]ELTCD: Extract of leaf of *Toona ciliata* in dichloromethane.
[3]II = Inhibition Index.
[4]CI =Classification Interval.
[5]Classification = Null: Included in the classification interval (CI < II < CI); Inhibitor: II <CI; Stimulant: II > CI.
or: II <CI; Stimulant: II > CI.

ulation, suppressor effects and/or deterrent effects. The application of pure azadirachtin deters landings of *B. tabaci*, and also reveals distinct behavioral modifications in response to azadirachtin characterized by the inhibition of probing (insect not moving across the leaf surface with the labium tip stationary on the leaf surface) and an increase in labial grooming (rapid movements of the legs across the labium) (Wen et al. 2009). In this context in our study, azadirachtin may have caused a stimulation of locomotion of *B. tabaci*, i.e., stimulating or accelerating movement, causing irritability and inducing dispersal. However, the behavioral effects of Meliaceae fraction extracts require further investigation.

Secondary metabolite extracts from Meliaceae may serve as a suitable future insect control substances potentially suitable for ecofriendly approaches for controlling *B. tabaci*. The present study revealed that the crude extracts and fractions from Meliaceae trees caused mortality of *B. tabaci* nymphs on tomato. However, the ovicidal effects of Meliaceae extracts on *B. tabaci* have yet to be elucidated. In addition, we recorded distinct behavioral modifications in response to fractions of Meliaceae extracts. Regarding their use on tomato, the ELTCD dichloromethane fraction reduced the number of *B. tabaci* settling on leaflets, and the ELTCD methanol and EBTPD dichloromethane fractions inhibited their oviposition.

Acknowledgments

The authors would like to thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for granting a scholarship to the first author and to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for granting a research grant to the third and fourth authors. We also thank Fabiana Fassis for technical assistance.

References Cited

Abbott, W. S, 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Abou-Fakhr Hammad, E. M., and Mcauslane H. J. 2006. Effect of *Melia azedarach* L. extract on *Bemisia argentifolii* (Hemiptera: Aleyrodidae) and its biocontrol agent *Eretmocerus rui* (Hymenoptera: Aphelinidae). Environ. Entomol. 35: 740-745.

Abou-Fakhr Hammad, E. M., Nemer, N. M., Hawi, Z. K., and Hanna, L. T. 2000. Response of the sweet potato whitefly, *Bemisia tabaci,* to the chinaberry tree (*Melia azedarach* L.) and its extracts. Ann. Appl. Biol. 137: 79-88.

Abou-Fakhr Hammad, E. M, Zournajian, H., and Talhouk, S. 2001. Efficacy of extracts of *Melia azedarach* L. callus, leaves and fruits against adults of the sweet potato whitefly *Bemisia tabaci* (Hom. Aleyrodidae). J. Appl. Entomol. 125: 483-488.

Baldin, E. L. L., Souza, D. R., Souza, E. S., and Beneduzzi, R. A. 2007. Controle de mosca-branca com extratos vegetais, em tomateiro cultivado em casa-de-vegetação. Hortic. Brasileira 25: 602-606.

Bartlett, M. S. 1937. Properties of sufficiency and statistical tests. Proc. R. Soc. Lond. Ser. A. 160: 268-282.

Bezerra-Silva, G. C. D., Vendramim, J. D., Silva, M. A., and Dias, C. T. S. 2010. Efeito de extratos orgânicos de Meliaceae sobre *Bemisia tabaci* (Gennadius) biótipo B em tomateiro. Arq. Inst. Biol. 77: 477-485.

Box, G. E. P., and Cox, D. R. 1964. An analysis of transformations. J. Royal Stat. Soc. 26: 211-252.

Champagne, D. E, Koul, O., Isman, M. B., Scudder, G. G. E., and Towers, G. H. N. 1992. Biological activity of limonoids from the Rutales. Phytochemistry. 31: 377-394.

Coudriet, D. L., Prabhaker, N., and Meyerdirk, D. E. 1985. Sweet potato whitefly (Homoptera: Aleyrodidae): Effects of neem-seed extract on oviposition and immature stages. Environ. Entomol. 14: 776-779.

Cubillo, D., Quijije, R., Larriva, W., Chacon, A., and Hilje, L. 1994. Evaluación de la repelencia de varias substancias sobre la mosca blanca *Bemisia tabaci* (Homoptera: Aleyrodidae). Man. Integr. Pla. 33: 26-28.

Cunha, U. S., Vendramim, J. D., Rocha W. C., and Vieira P. C. 2005. Potencial de *Trichilia pallida* Swartz (Meliaceae) como fonte de substâncias com atividade inseticida sobre a traça-do-tomateiro, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotrop. Entomol. 34: 667-673.

Cunha, U. S, Vendramim, J. D, Rocha, W. C, and Vieira, P. C. 2006. Frações de *Trichilia pallens* com atividade inseticida sobre *Tuta absoluta*. Pesq. Agropec. Brasileira 41: 1579-1585.

De Barro, P. J., Liu, S., Boykin, L. M., and Dinsdale, A. B. 2011. *Bemisia tabaci*: A statement of species status. Annu. Rev. Entomol. 56:1-19.

Fang, X., Di. Y. T., and Hao, X. J. 2011. The Advances in the limonoid chemistry of the Meliaceae family. Curr. Org. Chem. 15: 1363-1391.

Feng, R., and Isman, M. B. 1995. Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. Experientia 51: 831-833.

Ghazawy, N. A., Awad, H. H., and Abdel Rahman, K. M. 2010. Effects of azadirachtin on embryological development of the desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae). J. Orthopt. Res. 19: 327-332.

Heasley, B. 2011. Synthesis of limonoid natural products. Eur. J. Org. Chem. 2011: 19-46.

Kanokmedhakul, S., Kanikmedhakul, K., Prajuabsuk, T., Panichajakul, S., Panyamee, P., Prabpai, S., and Kongsaeree, P. 2005. Azadirachtin derivatives from seed kernels of *Azadirachta excelsa.* J. Nat. Prod. 68: 1047-1050.

Kogan, M., and Goeden, R. D. 1970. The host-plant range of *Lema trilineata daturaphila* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 63: 1175-1180.

Kumar, P, Poehling, H. M., and Borgemeister, C. 2005. Effects of different application methods of azadirachtin against sweet potato whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) on tomato plants. J. Appl. Entomol. 129: 489-497.

Liu, T. X., and Stansly P. A. 1995. Toxicity of biorational insecticides to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on tomato leaves. J. Econ. Entomol. 88: 564-568.

Mordue (Luntz), A. J., Morgan, E.D., and Nisbet, A. J. 2005. Azadirachtin, a natural product in insect control, pp. 117-134 *In* L. I. Gilbert, K. Iatrou and S. S.

Gill [eds.], Comprehensive molecular insect science. Elsevier, Oxford.

Morgan E. D. 2009. Azadirachtin, a scientific gold mine. Bioorg. Med. Chem. 17: 4096-4105.

Oiano Neto, J., Agostinho, S. M. M., Silva, M. F. G. F; Vieira P. C., Fernandes, J. B., Pinheiro, A. L., and Vilela, E. F. 1995. Limonoids from seeds of *Toona ciliata* and their chemosystematic significance. Phytochemistry 38: 397-401.

Prabhaker, N., Toscano, N. C., and Coudriet, D. L. 1989. Susceptibility of the immature and adult stages of the sweet potato whitefly (Homoptera: Aleyrodidae) to selected insecticides. J. Econ. Entomol. 82: 983-988.

Prabhaker, N., Toscano, N. C., and Henneberry, T. J. 1999. Comparison of neem, ureia, and amitraz as oviposition supressants and larvicides against *Bemisia argentifolii* (Homoptera: Aleyrodidae). J. Econ. Entomol. 92: 40-46,

Price, J. F., Schuster D. J., and Mcclain, P. M. 1990. Azadirachtin from neem tree (*Azadirachta indica* A. Juss.) seeds for management of sweet potato whitefly (*Bemisia tabaci* (Gennadius)) on ornamentals. Proc. Florida State Hortic. Soc. 103: 186-188.

Roel, A. R., and Vendramim, J. D. 1999. Desenvolvimento de *Spodoptera frugiperda* (J. E. Smith) em genótipos de milho tratados com extrato acetato de etila de *Trichilia pallida* (Swartz). Sci. Agric. 58: 581-586.

Roel, A. R., Vendramim, J. D., Frighetto R. T. S, and Frighetto, N. 2000a. Atividade tóxica de extratos orgânicos de *Trichilia pallida* (Swartz) (Meliaceae) sobre *Spodoptera frugiperda* (J. E. Smith). An. Soc. Entomol. Brasiliera 29: 799-808.

Roel, A. R., Vendramim, J. D, Frighetto, R. T. S, and Frighetto, N. 2000b. Efeito do extrato acetato de etila de *Trichilia pallida* (Swartz) (Meliaceae) no desenvolvimento e sobrevivência da lagarta-do-cartucho. Bragantia 59: 53-58.

SAS, Institute, SAS: User guide: versão 9.1. Cary, NC. 2002-2003.

Shapiro, S. S., and Wilk, M. B. 1965. An analysis of variance test for normality. Biometrika 52: 591-611.

Silva, M. A., Bezerra-Silva, G. C. D., Vendramim, J. D., and Mastrangelo, T. 2011. Inhibition of oviposition by neem extract:a behavioral perspective for the control of medfly. Florida Entomol. 95: 332-336.

Souza, A. P., and Vendramim J. D. 2000a. Atividade ovicida de extratos aquosos de meliáceas sobre a mosca-branca *Bemisia tabaci* (Gennadius, 1889) biótipo B em tomateiro. Sci. Agric. 57: 403-406.

Souza, A. P., and Vendramim, J. D. 2000b. Efeito de extratos aquosos de meliáceas sobre *Bemisia tabaci* biótipo B em tomateiro. Bragantia 59: 173-179.

Waterman P. G. 1993. Phytochemical diversity in the order Rutales, pp. 203-233 *In* K. Downum, J. T. Romeo and H. Stafford [eds.], Phytochemical potential of tropical plants. Plenum, New York.

Wen, J., Lin, K., Hou, M., Lu, W., and Li, J. 2009. Influence of foliar and systemically applied azadirachtin on host-plant evaluation behavior of the sweet potato whitefly, *Bemisia tabaci*. Physiol. Entomol. 34: 98-102.