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Botanical extracts: alternative control for silverleaf whitefly management in tomato

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ABSTRACT

Bemisia tabaci (Hemiptera: Aleyrodidae) biotype B is one of the most limiting pests of tomato crops in the world. Tomato yield is currently dependent on the use of pesticides, which are problematic to farmers, consumers and the environment. A promising alternative to reduce the harmful effects caused by the indiscriminated use of synthetic insecticides is the use of insecticides of botanical origin. This study aimed to evaluate the effect of 3% (w/v) aqueous extracts from different structures of thirteen botanical species on the behavior of *B. tabaci* biotype B adults, as well as insecticidal activity of such aqueous extracts on *B. tabaci* eggs, nymphs, and adults infesting tomato plants. A distilled water solution was used as a negative control, and thiamethoxam insecticide (18 g/100 L of water) as a positive control. Leaf extract of *Toona ciliata* was observed to have the most efficient inhibitory effect in tests of extracts on whitefly behavior. Furthermore, the use of leaf extract of *Toona ciliata* led to the most drastic reduction in the number of adults and eggs on tomato leaflets. Leaf extract of *Piper aduncum* led to the greatest observed ovicidal effect (78.00% of non-hatched nymphs); however it was not effective against nymphs and adults. The leaf extracts of *Trichilia pallida, Trichilia casaretti,* and *Toona ciliata* showed the highest control indexes (67.9, 60.3, and 55.1%, respectively). For adults mortality, *T. pallida* was the most effective (72.8%). Our results indicate that application of extracts of *T. pallida*, *T. ciliata*, and *T. casaretti* are promising strategies to manage *B. tabaci* biotype B on tomato.

Keywords: *Bemisia tabaci*, *Solanum lycopersicum*, Hemiptera, aqueous extracts.

RESUMO

Extratos botânicos: controle alternativo para o manejo de mosca-branca em tomateiro

Bemisia tabaci (Hemiptera: Aleyrodidae) biótipo B é uma das mais limitantes pragas do tomateiro no mundo. A produção do tomateiro é altamente dependente de inseticidas, ocasionando problemas ao produtor, consumidor e ambiente. Uma alternativa promissora para redução dos efeitos maléficos ocasionados pelo uso indiscriminado de inseticidas sintéticos são os inseticidas de origem botânica. Este trabalho objetivou avaliar o efeito de extratos aquosos a 3% (p/v) de diferentes estruturas de treze espécies botânicas no comportamento dos insetos adultos de *B. tabaci* biótipo B bem como sua atividade inseticida sobre ovos, ninfas e adultos do inseto em tomate. A água destilada foi utilizada como controle negativo e o inseticida tiametoxam (18 g/100 L de água) como controle positivo. O extrato à base de folhas de *Toona ciliata* foi o mais eficiente nos testes em que foram avaliados o efeito dos extratos sobre o comportamento da mosca-branca, diminuindo o número de insetos adultos e ovos em folíolos de tomateiro. O extrato de folhas de *Piper aduncum* apresentou o maior efeito ovicida (78,00% de ninfas não eclodidas), no entanto foi pouco efetivo sobre ninfas e adultos. Os extratos de folhas de *Trichilia pallida, Trichilia casaretti* e *Toona ciliata* apresentaram os maiores índices de controle (67,9; 60,3; 55,1%, respectivamente). Para adultos, *T. pallida* foi o mais eficiente (72,8%). Com base nos resultados, a aplicação dos extratos de *T. pallida*, *T. ciliata* e *T. casaretti* mostra-se promissora no manejo de infestações de *B. tabaci* biótipo B em tomateiro.

Palavras-chave: *Bemisia tabaci*, *Solanum lycopersicum*, Hemiptera, extratos aquosos.

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Tomato (*Solanum lycopersicum*) is one of the most important vegetables produced in Brazil and around the world, with a global production of approximately 161 million tons per year (FAOSTAT, 2014). The productivity of tomato crops is constantly threatened by pest attacks. Among the species most harmful to tomato, the silverleaf whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype B poses a particular threat in subtropical, tropical and temperate regions worldwide (Srinivasan *et al.*, 2012). *B. tabaci* infestation can lead to significant reduction in tomato plant yield and quality. Damage can be directly caused by sucking phloem sap, resulting in plant physiological disorders, such as leaf wilting and irregular ripening of the fruits; damage can be indirectly inflicted by excretion of honeydew, which can promote growth of black sooty mold fungi (*Capnodium* sp.), consequently interfering with photosynthesis and favoring viral infection (Firdaus *et al.*, 2012).

Tomato production is highly dependent on the use of pesticides. However, these products are generally considered harmful to the environment, producers and consumers (Broekgaarden *et al.*, 2011). The indiscriminated use of these conventional insecticides has been causing several problems, such as development of insect resistance to active ingredients, increasing environmental pollution and health risks (Forget *et al.*, 1993; Isman, 2006). Thus, it becomes necessary to reduce the amount of chemicals applied to crops and seek alternative methods to control *B. tabaci* biotype B that ensure high activity against target pests and lower impact on humans and the environment (Sayeda *et al.*, 2009).

Insecticides extracted from natural plant products are considered an important source of bioactive substances and are compatible with programs of integrated pest management (IPM) (Ateyyat *et al.*, 2009). This practice can be used jointly with other methods of pest control in efforts to maintain environmental balance because it leaves less chemical residue and causes low toxic effects on animals or humans (Isman, 2006; Morgan, 2009).

Studies with insecticide plants have evolved over the last decades, mostly on the Meliaceae family, especially the species *Azadirachta indica*. Several studies have shown the efficiency of using *A. indica*, *Trichilia pallida* and other meliaceous plants to control *B. tabaci* biotype B (Bezerra-Silva *et al.*, 2010; Bezerra *et al.*, 2012). Face of these positive results, a significant increase is expected in researches in this area, especially regarding their use on crops which require more attention in cultivation such as tomato; as well as the identification of new molecules for developing of bioinsecticides that can contribute to reduce the use of synthetic insecticides and their negative effects. Thus, the present study aimed to evaluate the behavioral effects and insecticidal activities of different plant extracts against *B. tabaci* biotype B infesting tomato plants.

MATERIAL AND METHODS

This research was carried out in a greenhouse (T= 28±4ºC; R.H.= 65±10%) and in a laboratory (T= 25±2ºC; R.H.= 60 ± 10%; 14:10-L:D) from 2011 to 2012.

Rearing of *B. tabaci* biotype B - Adult whiteflies were collected from a research colony of the Department of Crop Protection, from the São Paulo State University in Botucatu, São Paulo state, Brazil. The adults were maintained in a screen cage (2.5x2.5x2.0 m) covered with plastic sheet and shade cloth. The lateral and frontal parts were protected with white anti-aphid screens. For the colony maintenance, pots (20 L) containing soy, cabbage, and squash plants were placed into the cage. The plants were monitored on a weekly basis. The whiteflies (*B. tabaci* biotype B) were identified by Dr. Judith K. Brown, University of Arizona, USA. This identification was performed periodically through the cultivation of squash plants within the greenhouse, inducing the plants to express leaf silvering, a typical physiological disorder caused by the feeding of biotype B immature insects on this crop (Brown *et al.*, 1995). The colony was maintained free of insecticides spraying.

Harvest of biological material and preparation of extracts - Santa Clara cv. tomato seedlings were grown in polystyrene trays (128 cells) with recommended commercial substrate. The tomato seedlings were transplanted 20-30 days after sowing into 2 L plastic pots containing sterilized substrate and were fertilized following the culture recommendations. These plants were cultivated in another greenhouse free from insect infestation.

Thirteen species were evaluated in the current study: *Azadirachta indica*, *Chenopodium ambrosioides* (leaves + stem + inflorescence), *Mansoa alliacea* (leaves), *Mentha pulegium* (leaves), *Piper aduncum* (leaves), *Piper callosum* (leaves), *Pelargonium graveolens* (leaves), *Plectranthus neochilus* (leaves), *Ruta graveolens* (leaves), *Trichilia casaretti* (leaves), *Trichilia pallida* (leaves), *Toona ciliata* (leaves) and *Vitex agnus-castus* (leaves). A distilled water solution was used as a negative control, and thiamethoxam insecticide (18 g/100 L of water) as a positive control.

The plant species were chosen based on their historic of efficiency against some insect species, as well as economic and scientific interests.

After collection in 2011, the different plant structures of each plant were dried through air circulation at 40ºC for 48 h. The dried materials were triturated using a mill electric knife. The powders were stored separately in hermetically sealed containers (Baldin *et al.* 2007).

Aqueous extracts were prepared by mixing 0.9 g of powder with 30 mL of distilled water. Solutions were then kept under agitation for 24 h to facilitate the extraction of the compounds. The resulting suspensions were then filtered in voile fabric (5 layers) to produce 3% (w/v) aqueous extracts.

Effect of aqueous extracts on the behavior of adult insects - The bioassays were performed inside transparent plastic cages in laboratory. The cages had two free parts inside: one for the support of the glass vials (filled with distilled water and tomato leaflets), and another for the confinement of the insects. The support was made with a polystyrene plate (12x5x2 cm) containing two lateral orifices, to fit the glass vials (10 mL). This support was glued onto another polystyrene base (19x19x1.5 cm) with two more lateral orifices: one covered with voile fabric (aeration), and the other used to release the insects. The second part of the cage (for insect confinement) was composed of a transparent 2.5 L plastic container (14x15 cm) (Fanela *et al.*, 2012). The insects were collected with glass entomological aspirators (9x2.5 cm).

For each cage, two tomato leaflets were manually sprayed with treatments until the point of run-off (5 mL). Five minutes after spraying, leaflets were placed in glass vials containing distilled water (turgescence maintaining), and the vials were put in the base of the cage. Afterwards, 20 pairs (40 in total) of *B. tabaci* biotype B (1 to 2 days old) were introduced into the cage from the base. The total number of insects and deposited eggs on the leaflets were quantified 24 h after infestation. The experimental design was completely randomized with 5 replications and 15 treatments: 13 aqueous extracts, thiamethoxam insecticide (positive control) (18 g/100 L of water), and the negative control (distilled water). Each cage was considered one replication.

Insecticidal effects - Three experiments were carried out under controlled conditions (T= 25±2°C; R.H.= 65±10%; 14:10-L:D) in order to evaluate the insecticidal effect of contact with the aqueous extracts on whitefly eggs, nymphs and adults.

To obtain the necessary number of eggs for the study, 2 L pots of 30 to 40 days-after-emergence tomato plants were placed inside the rearing cage of whitefly for 24 h. After this period, adults were removed from the plants and the tomato plants were conducted to the laboratory for oviposition verification under microscope-stereoscope (up to 40x). Next, 30 viable eggs/leaflet were demarcated with *glitter* glue. Tomato leaves containing three leaflets with 30 viable eggs/leaflet were detached from the plants. These leaves were inserted inside plastic straws and placed in glass vials (9x2.5 cm) sealed with latex membrane. These vials were filled with distilled water to keep the turgescence of the leaflets. Treatments were then manually sprayed on the lower surface of the leaflets. During the experiment, the vials were placed in an appropriated tray (glassware).

The bioassay was carried out in completely randomized design with 15 treatments, 13 aqueous extracts (3% w/v), distilled water and thiamethoxam (18 g/100 L of water) and 3 replications (one per leaflet), totaling 90 eggs per treatment. The mortality was evaluated between 7 and 10 days after spraying (DAS) based on the percentage of non-hatched nymphs.

The methodology used to investigate ovicidal effect was also adopted for examining the effect on nymphs. When the nymph reached the second instar (N2), they were demarcated, in number of 30 per leaflet. The same treatments were sprayed on the lower surface of the leaflets. The mortality of nymphs was quantified at 3, 5, 7 and 9 DAS.

Aiming to evaluate adulticidal effect, tomato seedlings (15 to 20 days) were transferred to glass pipes (9x2.5 cm) containing moistened Bioplant® substrate. Two liter transparent plastic containers (26x10 cm), covered on top with voile fabric, were used for insect containment. The basal surfaces of these containers were composed of a Styrofoam plate (16x13x2.0 cm) with a central perforation (infestation, nebulization and subsequent fitting of the tomato seedling), and covered with black cardboard to facilitate the dead insects visualization. A nebulizer (adapted inhaler "ST SUPER-NS") was used as a sprayer agent to produce smaller drops.

To start the bioassay, adults (24 to 48 hours old) of *B. tabaci* biotype B (n= 40) were released from the central basal orifice of the Styrofoam plate, which was previously closed with voile fabric and tape. Five minutes after releasing, nebulization was performed with the nebulizer on the base for 30 seconds (period required for the equipment to fill the whole volume of the cage with the produced mist). After the mist disappeared (up to five minutes), glass pipes containing the tomato seedling were added inside the glass containers. The time intervals used in the current study were the same as those described by Fanela *et al*. (2012) in a previous study.

The experimental design was completely randomized with the same treatments mentioned for the ovicidal effect, 13 aqueous extracts (3% w/v), distilled water and thiamethoxam (18 g/100 L of water), and three replications. Each cage represented one replication. The counting of dead adults was performed at 24, 48 and 72 h after nebulization.

Statistical analysis - Two measures were used to assess the effect of the aqueous extracts on the behavior of *B. tabaci* biotype B: the inhibition indices of adults (II) and the oviposition deterrence index (DI) (adapted from Lin *et al*. 1990), following the formula: II or ID= 2G / (G+P) where G= number of insects or eggs counted on the treatment, and P= number of insects or eggs counted on the control. Based on the calculated indices and observed standard deviations, the classification intervals (CI) for the means of the treatments were determined using the formula: CI= $[(1 \pm t_{(n-1;\ \infty=0.05)}) \times (SD/n^{1/2})]$; where *t*= Student's t-test value at 5% probability, SD= standard deviation and n= number of replicates. Treatments were considered neutral when the values of their indexes were within the calculated CI, inhibitors when the values were lower than the calculated CI, and stimulants when values were higher than the calculated CI (Silva *et al*., 2012).

When necessary, the data about the insecticidal activity of the extracts were normalized by arc sine $(x/100)^{1/2}$ and $(x+0.5)^{1/2}$ transformations, then subsequently subjected to analysis of variance by F test, and the means compared by Tukey's test ($p<0.05$). All statistical analyses were performed by using SASM (Agri -System for Analysis and Mean Separation in Agricultural Experiments), Version 3.2.4 software. Calculation of the control efficiency of the extracts was carried out using the formula proposed by Schneider-Orelli (1947): MC(%) = [(Mortal.(%) in T – Mortal.(%) in C) / (100-Mortal.(%) in C)] * 100, where: MC(%)= corrected mortality in the control and T= mortality in the treatment. The mortality in the treatment with distilled water was used as control for this calculation.

RESULTS AND DISCUSSION

Effect of aqueous extracts on the behavior of adult insects - Twenty-four hours after spraying the extracts, an inhibitory effect was verified of the treatments *T. ciliata* (0.22), *P. neochilus* (0.33), *T. pallida* (0.42), *M. pulegium* (0.56), *P. callosum* (0.56), and *Trichilia casaretti* (0.58) on whitefly adults, when compared to the negative control (distilled water). The remaining treatments were classified as neutral. *T. ciliata* extract was the most efficient in this evaluation (Figure 1).

The extracts of *P. neochilus* (0.34), *P. callosum* (0.54), *T. pallida* (0.37), *T. ciliata* (0.23) and *T. casaretti* (0.44) were considered inhibitors of oviposition by

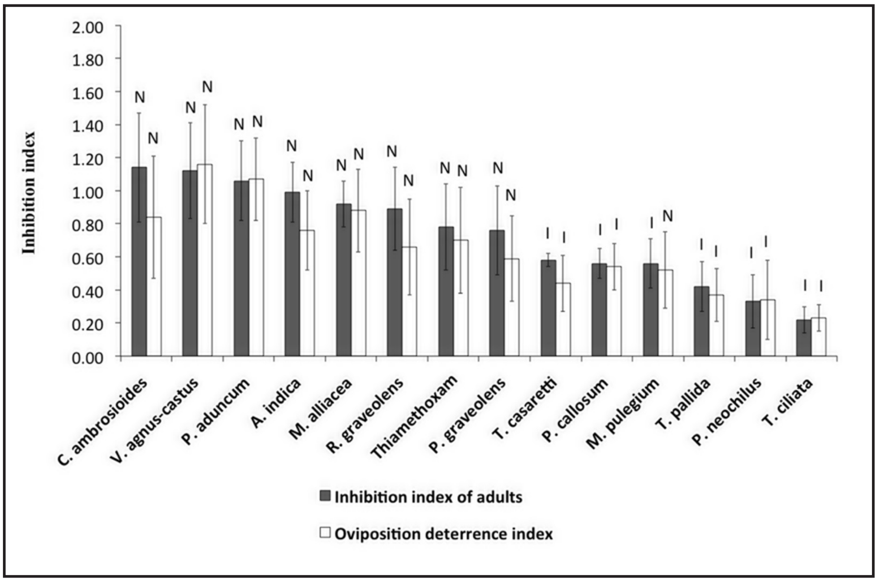

Figure 1. Inhibition caused by aqueous extracts and the thiamethoxam insecticide to oviposition and adults of *Bemisia tabaci* biotype B on tomato leaflets 24 h after spraying (inibição causada por extratos aquosos e o inseticida tiametoxam sobre a oviposição de adultos de *Bemisia tabaci* biótipo B em folíolos de tomateiro após 24 horas da pulverização) (T= 25±2°C; RH= 65±10%; 14:10-L:D). Classification: N= Neutral: comprising within classification interval (ICi<II<ICs); I= Inhibitor (II<ICi); E= Stimulant (II>ICs); Error bars ± SEM {classificação: N = Neutro: compreendendo dentro do Intervalo de classificação (ICi<II<ICs); I= Inibidor (II<ICi); E= Estimulante (II>ICs); Barras de erro ± MEP}. Botucatu, UNESP, 2011-2012.

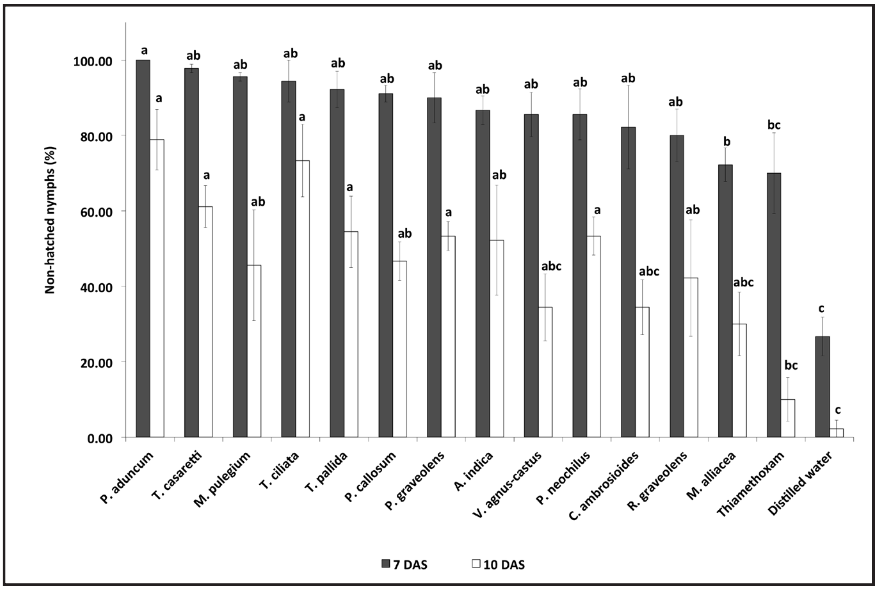

Figure 2. Mean percentage (±SE) of non-hatched *Bemisia tabaci* biotype B nymphs on sprayed tomato leaflets with different botanical extracts {percentagem média (±EP) de ninfas não eclodidas de *Bemisia tabaci* biótipo B em folíolos de tomateiro pulverizados com diferentes extratos vegetais} (T= 25±2°C; RH= 65±10%; 14:10-L:D). Means followed by the same letter do not differ by Tukey test (p>0.05). For statistical analysis, the data were transformed in arcsine $(x/100)^{1/2}$. Error bars ± SEM; DAS= days after spraying {médias seguidas da mesma letra não diferem entre si pelo teste de Tukey. Para análise estatística, os dados foram transformados em arcsen $(x/100)^{1/2}$. Barra de erros ± MEP; DAS= dias após pulverização}. Botucatu, UNESP, 2011-2012.

whiteflies. The remaining extracts were classified as neutral and did not differ from leaflets treated with distilled water (Figure 1).

Spraying with the meliaceous *T. ciliata*, *T. pallida*, *T. casaretti*, the lamiaceous *P. neochilus*, and the piperaceous *P. callosum* led tomato plants to be a less favorable host crop for the development of *B. tabaci* biotype B. While the effects of insecticidal plants on eggs, and mainly nymphs, are already well reported in the literature (Bezerra *et al*., 2010), the present results suggest inhibition mediated by volatile compounds, and it may contribute to the development of new strategies for the management of the whitefly on tomato crops.

The genus *Plectranthus* has several important phytochemical constituents, such as diterpenoids, essential oils and phenolics (Abdel-Mogib *et al*., 2002). Wellsow *et al*. (2006) tested the extracts of different species of *Plectranthus* against *Spodoptera littoralis* (Lepidoptera: Noctuidae), and observed antifeedant activity at 100 ppm for some plant species possibly due to the presence of diterpenoids.

The extracts of *Piper* species have insecticidal activity reported worldwide (Autran *et al*., 2009). Although this genus has many species that are known to have insecticidal activities against several insects, there are few studies on *P. neochilus* and *P. callosum* species reported in the literature.

Among the plants of the Meliaceae family, *A. indica* presents a larger range of studies regarding the behavior of *B. tabaci*. According to Baldin *et al*. (2007), among the aqueous extracts sprayed on tomato plants that did not stimulate colonization by *B. tabaci* biotype B, the extract of neem seeds and leaves stood out with means of adults and eggs below 0.60 and 0.50, respectively, differing from the treatment with distilled water. Quintela &Pinheiro (2009) also reported reduced silverleaf whitefly oviposition on leaves of common beans sprayed with aqueous extract of *A. indica*. However, in this study, the aqueous extract of neem seeds was classified as neutral for host selection. These differences may be attributed to the methodologies used in the studies such as preparation, concentration, and source of material.

The majority of the isolated limonoids from meliaceous present feeding deterrent properties. Studies about the mechanisms of feeding

Table 1. Mean mortality (±SE) and control efficiency of *B. tabaci* biotype B nymphs on tomato leaflets after spraying of aqueous extracts under different evaluation periods{mortalidade média (±EP) e eficiência de controle de ninfas de *B. tabaci* biótipo B em folíolos de tomateiro, após a aplicação dos extratos aquosos em diferentes períodos de avaliação} (T= 25±2°C; RH= 65±10%; 14:10-L:D). Botucatu, UNESP, 2011-2012.

Extracts	Mortality of nymphs (%)[†]				[‡]CE (%)
	3 DAS	5 DAS	7 DAS	9 DAS	
T. pallida	48.9 ± 7.29 a	53.3 ± 11.71 a	62.2 ± 7.78 a	72.2± 6.76 a	67.9
P. neochilus	40.0 ± 3.85 ab	47.7 ± 9.49 a	47.7 ± 9.49 a	51.1 ± 8.01 abc	43.4
M. pulegium	38.9 ± 5.88 ab	44.4 ± 7.29 a	46.6 ± 6.67 a	50.0 ± 5.09 abc	42.3
R. graveolens	28.9 ± 2.22 abc	32.2 ± 1.11 a	37.7 ± 4.01 a	40.0 ± 5.77 abc	30.7
T. ciliata	26.7 ± 6.67 abc	32.2 ± 1.11 a	54.4 ± 7.78 a	65.5 ± 5.88 ab	60.2
T. casaretti	22.2 ± 6.76 abc	34.4 ± 6.76 a	45.5 ± 2.22 a	61.1 ± 14.57 ab	55.1
Thiamethoxam	22.2 ± 9.88 abc	34.4 ± 12.37 a	42.2 ± 12.81 a	44.4 ± 12.37 abc	35.7
M. alliacea	18.9 ± 1.11 abc	26.6 ± 3.33 a	33.3 ± 1.92 ab	34.4 ± 2.22 abc	24.3
A. indica	16.7 ± 5.09 abc	37.7 ± 9.09 a	48.8 ± 12.81 a	54.4 ± 12.81 abc	47.4
V. agnuscastus	16.7 ± 8.39 bc	23.3 ± 12.02 a	27.7 ± 12.52 ab	28.8 ± 11.60 abc	17.9
C. ambrosioides	15.6 ± 2.94 bc	16.6 ± 3.33 ab	21.1 ± 1.11 ab	30.0 ± 1.92 abc	19.2
P. callosum	15.6 ± 5.56 bc	28.8 ± 12.37 a	37.7 ± 14.44 ab	45.5 ± 9.69 abc	37.2
P. aduncum	7.8 ± 2.22 cd	21.1 ± 6.76 a	21.1 ± 6.76 ab	24.4 ± 9.88 bc	12.8
P. graveolens	6.7 ± 1.92 cd	31.1 ± 1.11 a	40.0 ± 5.77 a	47.7 ± 9.09 abc	40.1
Distilled water	0.0 ± 0.00 d	0.0 ± 0.00 b	4.4 ± 2.94 b	13.3 ± 5.09 c	0.0
F	7.44^{*}	$4,70^{*}$	3.71^{*}	3.34^{**}	----
CV (%)	26.94	27,03	25.24	23.28	----

[†]The data were transformed in arcsine $(x/100)^{1/2}$. Means followed by the same letter in the column did not differ by Tukey's test (p>0.05); [‡]Control efficiency of the treatments calculated at the last evaluation period; DAS= days after spraying {os dados foram transformados em arcsen $(x/100)^{1/2}$. Médias seguidas pela mesma letra, na coluna não diferem entre si pelo teste de Tukey (p>0,05); [‡]Eficiência de controle dos tratamentos calculada no último período de avaliação; DAS= dias após pulverização}.

inhibitors demonstrated that the inhibition or reduction of feeding is due to either the inactivation of the function of certain chemoreceptors or the stimulus of specific deterrent receptors located medially or laterally in sensilla styloconicas (Li, 1999). The species *T. ciliata* is known as a source of cedrelone and toonacillin limonoids (Oiano-Neto *et al*., 1998). Toonacillin had insecticidal and antifeedant activity in *S. litura* (Govindachari *et al*., 1995). There are several reports regarding the biological properties of plant species that belong to the genus *Trichilia*. In *T. pallida*, three new tetranortriterpenoids were described, as well as two compounds already related in the literature, hirtin and diacetyl-hirtin (Simmonds *et al*., 2001). Xie *et al*. (1994) observed the growth-regulatory effects and antifeedant from the hirtina limonoid of *Trichilia hirta* on two species of Lepidoptera.

The presence of these secondary metabolites may have influenced the behavior of adult *B. tabaci* biotype B, thus reducing the number of insects and eggs in *T. ciliata* and *T. pallida* treatments. In the case of *T. casaretti*, the major chemical constituents have not been described in the literature yet.

Insecticidal effects - Regarding the ovicidal effect at 7 DAS, all treatments differed from the negative control, except for thiamethoxam. The extracts of *P. aduncum*, *T. ciliata*, *T. casaretti*, *T. pallida*, *P. graveolens* and *P. neochilus* showed the highest ovicidal effect (Figure 2). At 10 DAS, there was no difference between this synthetic insecticide and the treatments *M. alliacea*, *C. ambrosioides* and *V. agnus*-castus. The percentages of non-hatched nymphs at 10 DAS varied from 78.9% (*P. aduncum*) to 2.2% (distilled water).

We observed that some nymphs presented complete embryonic development and managed to break through the egg chorion, consequently having hatched. However, they died with the body partially adhered to the egg. This was often verified in the thiamethoxam treatment, in which no ovicidal action was observed. The mean number of dead nymphs in this situation was 23.67; nevertheless, 78.9% of the nymphs hatched but died adhered to the eggs. Prabhaker *et al*. (1999) also reported similar observations upon spraying an oil-based formulation of solanaceous *Nicotiana gossei* and the insecticide amitraz on the eggs of *B. tabaci* biotype B. The same authors reported that possibly the death of the nymphs occurred due to the residues of the extracts deposited on the chorion, which may also explain the results obtained in the present study.

Considering only the embryonic period, the data presented here show that the extracts examined in this study were more efficient than those tested by Bezerra-Silva *et al*. (2010). The authors observed higher control efficiency by using extracts of *T. pallida* (21.01%) on eggs of the same whitefly in tomato; however the authors used ethanolic

Table 2. Mean mortality (±SE) and control efficiency of adult *B. tabaci* biotype B on tomato leaflets, after application of aqueous extracts under different evaluation periods {mortalidade média (±EP) e eficiência de controle de adultos de *B. tabaci* biótipo B em folíolos de tomateiro, após a aplicação dos extratos aquosos em diferentes períodos de avaliação} (T= 25±2°C; R.H.= 65±10%; 14:10-L:D). Botucatu, UNESP, 2011-2012.

Extracts	**Mortality of adults (%)**†			**C.E.**‡ **(%)**
	24 h	**48 h**	**72 h**	
T. pallida	62.5 ± 0.88 a	66.6 ± 7.12 a	75.8 ± 10.64 a	72.8
T. casaretti	47.5 ± 0.92 ab	56.6 ± 10.14 ab	61.6 ± 9.28 a	55.3
C. ambrosioides	45.0 ± 0.89 ab	51.6 ± 1.67 ab	58.3 ± 3.63 ab	51.4
M. pulegium	40.0 ± 0.84 ab	51.6 ± 9.61 ab	61.6 ± 9.39 a	55.3
Thiamethoxam	40.0 ± 0.87 ab	54.1 ± 12.44 ab	62.5 ± 11.81 a	56.3
R. graveolens	37.5 ± 0.93 abc	42.5 ± 13.23 ab	45.8 ± 13.72 ab	36.8
T. ciliata	35.0 ± 0.89 abc	40.0 ± 11.55 ab	45.0 ± 11.46 ab	35.9
V. agnus-castus	30.0 ± 0.70 abc	31.6 ± 3.00 ab	45.0 ± 3.82 ab	35.9
P. graveolens	29.1 ± 0.95 abc	45.0 ± 8.66 ab	47.5 ± 7.22 ab	38.8
M. alliacea	27.5 ± 0.85 abc	33.3 ± 6.82 ab	39.1 ± 8.46 ab	29.1
A. indica	27.5 ± 0.88 abc	31.6 ± 7.12 ab	42.5 ± 10.64 ab	33.0
P. callosum	25.0 ± 0.87 abc	34.1 ± 3.00 ab	39.1 ± 0.83 ab	29.1
P. aduncum	22.5 ± 0.84 bc	31.6 ± 2.20 ab	37.5 ± 6.61 ab	27.1
P. neochilus	22.5 ± 0.85 bc	27.5 ± 6.61 ab	32.5 ± 9.01 ab	21.3
Distilled water	9.1 ± 1.00 c	14.1 ± 6.01 b	14.1 ± 6.01 b	0.0
F	3.76*	2.06*	2.81*	----
CV (%)	22.40	25.58	22.96	----

†The data were transformed in arcsine $(x/100)^{1/2}$. Means followed by the same letter in the column did not differ by Tukey's test (p>0.05); ‡Control efficiency of the treatments in the last evaluation period {médias seguidas da mesma letra, na coluna não diferem entre si pelo teste de Tukey (p>0,05); ‡Eficiência de controle dos tratamentos calculada no último período de avaliação}.

extract, while the aqueous extract was used in the present study.

In general, extract of *P. aduncum* resulted in the greatest ovicidal effect. However, there are no studies with this species regarding the control of *B. tabaci* biotype B. According to Silva *et al*. (2007), this plant can be used to control several pests, as it contains the secondary metabolite dillapiol, which has a recognized insecticidal effect and synergistic activity.

The extracts of *T. pallida*, *T. ciliata* and *T. casaretti* were the most efficient in controlling whitefly nymphs up to 9 DAS. These treatments differed from the control and had absolute values higher than the thiamethoxam treatment. The *T. pallida* extract always revealed the best indexes, highlighted by the last evaluation in which 21.67 dead nymphs were observed (72.2% of total), and only 4.00 (13.3% of total) were observed in the control during the same period. These three extracts revealed efficiencies ranging between 55.1% to 67.9%. For thiamethoxam, which was expected to have the greatest value, the efficiency was 35.7% (Table 1). The low efficiency of the synthetic insecticide may be related to genetic variability that exists among whitefly populations from different regions of the country, which implies in a higher or lower susceptibility to insecticides, including neonicotinoids (Silva *et al*., 2009). Bezerra-Silva *et al*. (2010) observed that the organic extract of *T. pallida* was the most effective with control efficiency higher than 70.0%.

The extracts of *A. indica* are described as extremely effective botanical insecticides, and its activity is related to the presence of azadirachtin tetranortriterpenoids (Pavela, 2007). Kumar & Poehling (2007) analyzed the direct and residual toxicity of different products, including NeenAzal-T/S (commercial insecticide), on *B. tabaci*, reporting nymphal mortality of 100% at 9 DAS. However, in the current study, the nymphal mortality of extract from *A. indica* did not differ from the negative control (distilled water). One possible explanation may be the quantity of the azadirachtin active ingredient existent in the neem aqueous extract.

Treatments with the extracts of *T. pallida*, *M. pulegium*, *T. casaretti*, and thiamethoxam caused the greatest mortality in *B. tabaci* adults at 72 h after nebulization. The *T. pallida* extract had the most efficient control and was also the only treatment that differed from the distilled water in all of the evaluations (62.5%, 66.6% and 75.8% of mortality, respectively), achieving an average of efficiency control of 72.8% after 72 h (Table 2). The insecticidal characteristic due to the metabolites found in *T. pallida* (Simmonds *et al*., 2001) may be the reason why the species also led to the highest efficiency to control adult insects of any of the treatments. The result was also higher than the synthetic insecticide, which has recognized bioactivity effect on *B. tabaci* biotype B (Villas Bôas, 2005).

There are few studies with the species *M. pulegium* and *T. casaretti* aiming to control *B. tabaci* biotype B adults; however, there are studies that indicate the insecticidal potential of these two plant species on the other arthropods. Bogorni &Vendramim (2005) reported that the extracts of *T. pallida* and *T. pallens* were more efficient, even though the extract of *T. casaretti* also affected the development of *S. frugiperda* in corn. Cetin *et al*. (2006) evaluated the larvicidal activity of organic extracts on *Culex pipiens* from five plants of the Lamiaceae family, including *M. pulegium*. They have concluded that all tested plants presented a high insecticidal activity against the mosquito larvae.

According to the results obtained in the current study, it can be concluded that the application of aqueous extracts from *T. ciliata*, *P. neochilus*, *T. pallida*, *P. callosum* and *T. casaretti* reduce the colonization and oviposition of *B. tabaci* biotype B in tomato crop. Moreover, the extracts of *T. ciliata*, *T. casaretti* and *T. pallida* are highly effective on the control of eggs and nymphs of the insect. Although further studies aiming substances' isolation and use standardization are still necessary, the presented findings demonstrate a great potential for these botanical species to be used as natural insecticide against whitefly or in conjunction with other control strategies that can contribute to reduce the use of synthetic insecticides on tomato crop.

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