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INSECTICIDAL ACTIVITY OF 1,4-DIHYDROPYRIDINES AGAINST BEMISIA TABACI MEAM1 (GENN.) (HEMIPTERA: ALEYRODIDAE)

ATIVIDADE INSETICIDA DE 1,4-DIIDROPIRIDINAS CONTRA BEMISIA TABACI MEAM1 (GENN.) (HEMIPTERA: ALEYRODIDAE)

ACTIVIDAD INSECTICIDA DE LAS 1,4-DIHIDROPIRIDINAS CONTRA BEMISIA TABACI MEAM1 (GENN.) (HEMIPTERA: ALEYRODIDAE)

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Thaís Aparecida dos Santos Oliveira[1], Yan Ramalho Robles[2], Aline Marques Pinheiro[3], Felipe Savieto Furquim de Souza[4], Edson Luiz Lopes Baldin[5], Antônio Eduardo Miller Crotti[6], Vinicius Fernandes Canassa[7]

ABSTRACT
The silverleaf whitefly (Bemisia tabaci) is a pest that feeds on a wide range of herbaceous plants' phloem. This insect can cause direct damage to the hosts and transmits over 350 different species of viruses to the plants, reducing the crop productivity up to 100% in some cases. The main current strategy for pest control is the application of chemical pesticides, but the emergence of resistance to the pesticides due to prolonged and extensive use has been reported. In this study, a series of 1,4-dihydropyridine (1,4-DHPs) was synthesized through a one-pot multicomponent reaction and evaluated for their in vivo insecticidal potential against B. tabaci MEAM1 nymphs. Among the 24 compounds tested, at a concentration of 5 mg/L, compounds 10 (ethyl 2,7,7-trimethyl-4-(3-nitrophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate), 15 ethyl 4-(furan-2-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate), 16 (ethyl 2,7,7-trimethyl-5-oxo-4-(thiophen-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate), and 18 (diethyl 4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) displayed control efficiencies twice as high as that of the pyriproxyfen at 100 g/L), which was used as the positive control.

Keywords: *Dihydropyridines*. Hantzsch Reaction. Multicomponent Reaction. Silverleaf Whitefly.

RESUMO
A mosca-branca (Bemisia tabaci) é uma praga que se alimenta do floema de uma ampla variedade de plantas herbáceas. Este inseto pode causar danos diretos às plantas

[1] Dr. in Chemistry. Universidade de São Paulo. São Paulo, Brazil. E-mail: thaisoliveira@usp.br
[2] Undergraduate student in Chemistry. Universidade de São Paulo. São Paulo, Brazil.
E-mail: yanrobles@usp.br
[3] Master's degree in Agronomy. Universidade Estadual Paulista Júlio de Mesquita Filho. São Paulo, Brazil.
E-mail: aline.pinheiro@unesp.br
[4] Master's student in Agronomy. Universidade Estadual Paulista Júlio de Mesquita Filho. São Paulo, Brazil.
E-mail: felipe.savieto@unesp.br
[5] Dr. in Entomology. Universidade Estadual Paulista Júlio de Mesquita Filho. São Paulo, Brazil.
E-mail: edson.baldin@unesp.br
[6] Dr. in Chemistry. Universidade de São Paulo. São Paulo, Brazil. E-mail: millercrotti@ffclrp.usp.br
[7] Dr. in Agronomy. Universidade Estadual Paulista Júlio de Mesquita Filho. São Paulo, Brazil.
E-mail: vfcanassa@hotmail.com

hospedeiras e transmitir mais de 350 espécies diferentes de vírus, reduzindo a produtividade agrícola em até 100% em alguns casos. A principal estratégia atual de controle é a aplicação de pesticidas químicos, mas o surgimento de resistência devido ao uso prolongado e intensivo tem sido relatado. Neste estudo, uma série de 1,4-diidropiridinas (1,4-DHPs) foi sintetizada por meio de uma reação multicomponente em um único passo (one-pot) e avaliada quanto ao seu potencial inseticida in vivo contra ninfas de B. tabaci MEAM1. Entre os 24 compostos testados, na concentração de 5 mg/L, os compostos 10, 15, 16 e 18 apresentaram eficiências de controle duas vezes maiores do que o piriproxifeno a 100 g/L, usado como controle positivo.

Palavras-chave: *Diidropiridinas*. Reação de Hantzsch. Reação Multicomponente. Mosca-Branca.

RESUMEN

La mosca blanca de los invernaderos (Bemisia tabaci) es una plaga que se alimenta del floema de una amplia variedad de plantas herbáceas. Este insecto puede causar daños directos a los hospedadores y transmitir más de 350 especies diferentes de virus a las plantas, reduciendo la productividad de los cultivos hasta en un 100% en algunos casos. La principal estrategia actual para el control de esta plaga es la aplicación de pesticidas químicos; sin embargo, se ha informado de la aparición de resistencia debido al uso prolongado y extensivo de estos compuestos. En este estudio, se sintetizó una serie de 1,4-dihidropiridinas (1,4-DHPs) mediante una reacción multicomponente en un solo paso (one-pot) y se evaluó su potencial insecticida in vivo contra ninfas de B. tabaci MEAM1. Entre los 24 compuestos ensayados, a una concentración de 5 mg/L, los compuestos 10 (etil 2,7,7-trimetil-4-(3-nitrofenil)-5-oxo-1,4,5,6,7,8-hexahidroquinolina-3-carboxilato), 15 (etil 4-(furano-2-il)-2,7,7-trimetil-5-oxo-1,4,5,6,7,8-hexahidroquinolina-3-carboxilato), 16 (etil 2,7,7-trimetil-5-oxo-4-(tiofen-2-il)-1,4,5,6,7,8-hexahidroquinolina-3-carboxilato) y 18 (dietil 4-(4-metoxifenil)-2,6-dimetil-1,4-dihidropiridina-3,5-dicarboxilato) mostraron eficiencias de control dos veces superiores a las del piriproxifeno a 100 g/L, que se utilizó como control positivo.

Palabras clave: *Dihidropiridinas*. Reacción de Hantzsch. Reacción Multicomponente. Mosca Blanca de los Invernaderos.

1 INTRODUCTION

Bemisia tabaci Middle East-Asia Minor 1 (MEAM1) Genn. (Hemiptera: Aleyrodidae), also known as silverleaf whitefly, is an insect that lives usually in herbaceous plants, feeding from their phloem (DE BARRO et al., 2011). *Bemisia tabaci* diet causes direct damage to various types of crops such as cotton (SHUKLA et al., 2016), cabbage (SANTIAGO-ÁLVAREZ et al., 2006), potatoes (SANTIAGO-ÁLVAREZ et al., 2006), lettuce (NG & CHEN, 2011), eggplant (LEITE et al., 2003), tomato (CALVO et al., 2012), and soybeans (PADILHA et al., 2021), besides many others (ABUBAKAR et al., 2022). Besides causing direct damage to its hosts, the silverleaf whitefly's feeding is responsible for the transmission of more than 350 species of viruses, some capable of reducing the crop productivity by 20 to 100% (SANI et al., 2020). *Bemisia tabaci* is considered a pest capable of causing substantial damage and economic losses.

Numerous techniques for managing the population growth of silverleaf, referred to as whitefly management (WFM) strategies, have been documented. These strategies include a variety of methods, such as the application of diverse mixtures: botanical extracts (ATEYYAT et al., 2009; ZHANG et al., 2004), animal waste (SINGH et al., 2021), and ash (SHAILAJA, 2012) to crops, as well as mechanical and biological methods. Nevertheless, many cultural strategies are either suitable only for small-scale farmers or lack robust scientific validation (ABUBAKAR et al., 2022). Additionally, the biological approach may yield inconsistent results due to the numerous factors and parameters that influence the efficacy of biological control agents (ABUBAKAR et al., 2022; KIDANE et al., 2015). Chemical pesticides are the most reliable method for the control of *B. tabaci*, and most commonly include pyriproxyfen, buprofezen, spiromesifen, spirotetramat, anthranilic diamides, cyantraniliprole, and chlorantraniliprole (HOROWITZ et al., 2020). However, the application of these chemicals is associated with adverse effects on both human health and ecological safety (KIM et al., 2017). Furthermore, the persistent use of these substances in increasing amounts has resulted in the emergence of resistance in *B. tabaci* (HOROWITZ et al., 2020; SHAH et al., 2020). Consequently, the development of insecticide resistance makes the silverleaf management a substantial challenge.

The 1,4-dihydropyridine nucleus (1,4-DHP) is present in the structure of several compounds that present pharmacological activities, such as bronchodilator (WEI et al., 2015), anticonvulsant (WEI et al., 2015), hypotensive (MAGER et al., 1992; ROVNYAK et al., 1992), and calcium channel blockage (MIRI et al., 2006). 1,4-DHP compounds are also known for their ability to reverse multidrug resistance (CAROSATI et al., 2012). In addition, several compounds of this class exhibit diverse biological activities (NUNEZ-VERGARA et al., 1997). For example, recent studies demonstrated that 1,4-DHPs display *in vitro* antileishmanial and antitoxoplasmal activities (OLIVEIRA et al., 2025; OLIVEIRA et al., 2024). However, despite the wide variety of biological activities, the insecticidal activity of 1,4-DHPs is still little exploited in the literature.

In this scenario, this study aimed to investigate the toxicity of a series of synthetic 1,4-DHPs obtained via Hantzsch reaction in 2^{nd} instar nymphs of *B. tabaci* MEAM1.

2 METHODOLOGY

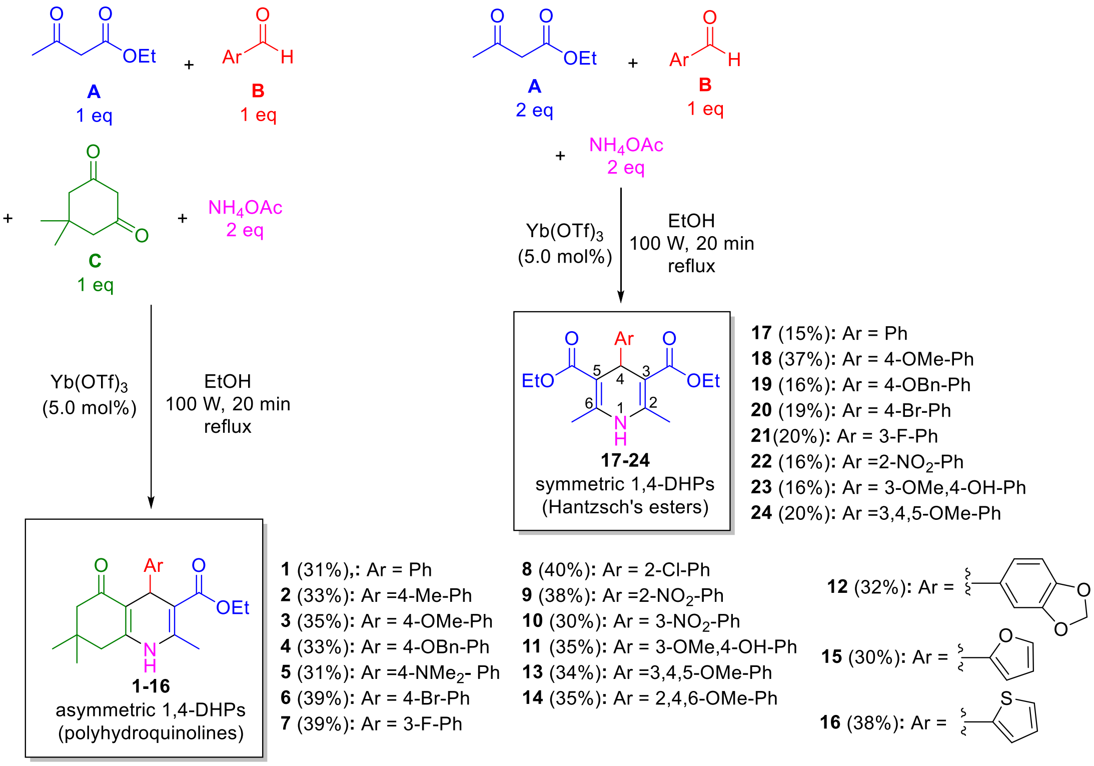
2.1 SYNTHESIS OF 1,4-DIHYDROPIRIDINES 1-24

The 1,4-dihydropyridines **1-24** were synthesized according to the multicomponent one-pot methodology previously described in the literature, with some modifications (Figure 1) (WANG et al., 2005). For the synthesis of asymmetric 1,4-DHP's (**1-16**), 2.0 mmol of dimedone (Aldrich), 2.0 mmol of ethyl acetoacetate (Aldrich), and 0.06 g (5.0 mol%) of ytterbium triflate (Aldrich) were added to a 15-mL round-bottom flask containing 5.0 mL of ethanol. Subsequently, 2.0 mmol of an aromatic aldehyde (Aldrich) and 2.0 mmol of ammonium acetate (Scientific Exodus) as a source of nitrogen were added to the flask, which was coupled to a reflux condenser and set to a CEM Discover microwave reactor (CEM Corp, Matthews, NC). All reagents were added at room temperature. The reaction mixture was taken to the microwave reactor, where it was maintained for 20 min at a fixed power of 100 W in an open vessel system configuration. The symmetric 1,4-DHPs (**17-24**) were obtained following a similar procedure, in which 2 eq. (4.0 mmol) of ethyl acetoacetate was used in place of 1 eq of ethyl acetoacetate and 1 eq of dimedone (Figure 1).

The starting material consumption and the product formation were monitored by thin-layer chromatography using a mixture of hexane: ethyl acetate 3:1 (v/v) as a mobile phase. After the reaction ended, compounds **1-24** were separated from the reaction mixture by vacuum filtration and further purified by recrystallization in ethanol. Compounds **1-24** were obtained in yields ranging from 15% to 40% (Figure 1) and identified based on their ^{1}H and ^{13}C NMR and mass spectrometry data, as previously reported (OLIVEIRA et al., 2025). Compounds **1-16** were evaluated as mixtures of enantiomers.

Figure 1

*Synthesis of 1,4-DHPs **1-14***



2.2 INSECTICIDAL ASSAY

The tests were carried out at the Laboratory of Plant Resistance to Insects and Insecticide Plants (LARESPI), located in the Department of Plant Protection (22° 50' 40" S 48° 26' 05" W) at the FCA/UNESP in the city of Botucatu, SP, during September 2022.

The tests with *B. tabaci* MEAM1 were carried out in the laboratory and a greenhouse, in a completely randomized design, with 25 treatments and five replicates, each replicate consisting of a leaflet with 20 nymphs (2nd instar) of *B. tabaci* MEAM1, totaling 100 nymphs per treatment. The rearing of *B. tabaci* MEAM1 was maintained in a greenhouse (3.0 × 3.0 × 2.5 m), covered with shade (30%), and closed laterally with an anti-aphid screen (200 mesh). For the maintenance of insects, cabbage (*Brassica oleracea* var. acephala L.), soybean (*Glycine max* L. Merrill), cotton (*Gossypium hirsutum* L.), and tomato (*Solanum lycopersicum* L.) plants were grown in 2.5 L plastic pots.

Soybean cultivar 'NA 5909' seeds were sown in plastic pots (0.70 L) containing Tropstrato® substrate (Vida Verde Indústria e Comércio de Insumos Orgânica Ltda, Mogi Mirim, São Paulo, Brazil). The pots were placed in a greenhouse, free from insect infestation, and received the other cultural practices normally recommended for the crop. Soybean plants with the first fully developed trifoliate leaf (0.7 L pots) were conditioned in the *B. tabaci* MEAM1 rearing, for oviposition by whitefly adults. After three days, the pots were removed and taken to a greenhouse until the eggs hatched and the nymphs reached the second instar, which were demarcated with the aid of a magnifying glass and glitter glue, and then sprayed with the different treatments. Five plants were used per treatment, and each plot consisted of a plant (leaflet) marked with 20 nymphs. In sequence, a single spraying was carried out using a professional airbrush (Model BD134K), coupled to an air compressor adjusted to provide a pressure of 0.5 bar. At the time of spraying, the plants were at stage 12, according to the BBCH scale (VARIANI, 2001). Compounds **1-24** were solubilized in Tween 20 and tested at a concentration of 5 mg/L. Tiger 100 EC® (pyriproxyfen 100 g/L) was used as a standard insecticide (positive control).

After spraying, the pots were kept in a greenhouse. The evaluation of nymphs was performed daily during seven consecutive days after application by counting live nymphs. During the evaluation period, the possible phytotoxicity of the products on the leaflets was also verified.

The effectiveness of the products was determined using the Abbott formula (NAKANO et al., 1981), as described below:

$$\%E = \left(1 - \frac{1}{C}\right) x\, 100 \qquad (1)$$

Where: %
E = Control effectiveness;
C = number of live *B. tabaci* nymphs in the control;
I = number of live *B. tabaci* nymphs in the evaluated treatment.

2.3 STATISTICAL ANALYSIS

The data obtained were subjected to analysis of variance by the F test. Normality was verified using the Shapiro-Wilk test and homogeneity using the Levene test. When significance was verified in the treatment effects, the Fisher LS-Means Test was used, adjusted for Tukey ($P \leq 0.05$) to compare means. For the analysis, the PROC MIXED-SAS 9.2 statistical package was used (INSTITUTE, 2011).

3 RESULTS AND DISCUSSION

Table 1 illustrates the results and the control efficiency obtained during the 7-day treatment with the 1,4-DHPs **1-24** at a concentration of 5 mg/L against *Bemisia tabaci* MEAM1 nymphs.

Compounds **1-9** and **11** displayed lower efficacy compared to the positive control (pyriproxyfen, 16.37%), whereas compounds **17**, **22**, and **23** had efficacies similar to the positive control. On the other hand, all the other 1,4-DHPs tested were more effective against *B. tabaci* nymphs than the positive control. The most effective compounds were **10** (34.38%), **15** (30.38%), **16** (34.83%), and **18** (34.83%).

The 1,4-DHP scaffold is present in many compounds whose biological activities have been explored. Recently, the potential of 1,4-DHPs derivatives obtained by the Hantzsch synthesis as agrochemicals has been addressed (PARTHIBAN & MAKAM, 2022). These nitrogen-containing molecules, although not directly derived from nicotine, possess structural resemblance to the neonicotinoid class, whose insecticidal properties are well-established in the literature (GUPTA et al., 2019). Studies report the insecticidal activity of DHPs against *Aphis medicaginis*, *Aphis craccivora*, *Nilaparvata lugens*, *Brevicoryne brassicae*, and many others, which can be considered pests capable of damaging different types of crops (PARTHIBAN & MAKAM, 2022). However, to date, data on the insecticidal activity of 1,4-DHPs are unreported in the literature.

Noteworthy, the insecticide pyriproxyfen, at a concentration of 100 g/L, had an efficiency of only 16.37% (e.g., the efficiency of pyriproxyfen to reduce the number of live *B. tabaci* nymphs compared to the positive control group was low). In other studies performed by our group, pyriproxyfen at 100 g/L caused 100% mortality of *Bemisia tabaci* biotype B adults (BALDIN et al., 2018; HE et al., 2013). This may indicate that the *B. tabaci* biotype used in this study is resistant to pyriproxyfen.

Among the 24 1,4-DHPs tested, 14 showed higher control efficacy than the positive control. All the symmetrical compounds (the Hantzsch esters) **17-24** were more effective against *B. tabaci* MEAM1 as compared to pyriproxyfen, whereas most (but not all) of the asymmetrical 1,4-DHPs (polyhydroquinoline) were less effective than the positive control. On the other hand, the polyhydroquinolines **10**, **15**, and **16,** alongside the Hantzsch ester **18,** were two times more effective than the positive control.

Compounds **15** and **16** display a heteroaromatic substituent at C4. On the other hand, compound **10** displayed a 3-nitro group at the aromatic ring, whereas compound **18** displayed a 4-methoxy group. These results could indicate that the presence of the 4-methoxyphenyl, 2-furanyl, 2-tiophenyl, and 3-nitrophenyl groups may play a key role in the activity of 1,4-DHPs against *Bemisia tabaci* MEAM1. However, further studies are needed to better understand the structure-insecticidal activity relationships of 1,4-DHPs against *B. tabaci* MEAM1.

Table 1

*Number of live Bemisia tabaci MEAM1 nymphs (mean ± SD) and control efficiency (%) after 7-day exposure to treatments with compounds **1-24** at 5 μL/mL*

	Bemisia tabaci	
Treatment	**Number of live individuals[a]**	**% control efficiency[b]**
Witness	19.03 ± 0.48 a	---
1	17.14 ± 0.80 ab	9.91
2	17.31 ± 0.73 ab	9.01
3	17.26 ± 0.38 ab	9.31
4	17.66 ± 0.51 ab	7.21
5	16.91 ± 0.72 ab	11.11
6	17.91 ± 0.86 ab	5.86
7	16.37 ± 0.52 ab	13.96
8	17.26 ± 0.48 ab	9.31
9	17.31 ± 1.21 ab	9.01
10	12.49 ± 1.20 b	34.38
11	18.43 ± 0.48 ab	3.15
12	14.34 ± 0.83 ab	24.62
13	14.34 ± 0.70 ab	24.62
14	13.94 ± 1.14 ab	26.73
15	12.49 ± 2.28 b	34.38
16	12.40 ± 1.39 b	34.83
17	15.63 ± 1.14 ab	17.87
18	12.40 ± 1.11 b	34.83
19	14.66 ± 0.77 ab	22.97
20	14.60 ± 1.44 ab	23.27
21	14.46 ± 1.67 ab	24.02
22	15.63 ± 1.07 ab	17.87
23	15.66 ± 1.15 ab	17.72
24	15.11 ± 1.30 ab	20.57
Pyriproxyfen	15.91 ± 0.73 ab	16.37

P	<0.0001

[a]Means followed by the same letter in the column do not differ by Tukey's adjusted LS-means test ($P \geq 0.05$); [b]To calculate the efficiency, the Abbott formula was used (NAKANO et al., 1981).

4 CONCLUSION

The polyhydroquinolines **10**, **15**, and **16**, and the Hantzsch ester **18,** displayed higher efficacy than pyriproxyfen (10 g/L) against *B. tabaci* MEAM1 nymphs at a concentration of 5 mg/L. These preliminary results indicated that 1,4-DHPs are a promising class of alternative insecticides against *B. tabaci* MEAM1 that can be obtained through an attractive methodology that requires only a synthetic step, employs mild and green conditions, and has short time reactions, which deserve to be deeper investigated.

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