

SELECTIVITY OF INSECTICIDES TO *Encarsia hispida* (Hymenoptera: Aphelinidae)¹

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ABSTRACT - The use of non-selective insecticides in agricultural production can reduce or eliminate biological agents that regulate insects that are undesirable to humans in production processes. Here, the toxicity of synthetic products on the parasitoid *Encarsia hispida* was evaluated, the host of which is the whitefly, *Bemisia tabaci* biotype B, found on cotton plants. The study was carried out using bioassays, in a completely randomized design in a factorial scheme. Pupae and adults of *E. hispida* were used in the bioassays, which had been sprayed with various chemicals (thiamethoxam, deltamethrin, imidacloprid, and pyriproxyfen) at different concentrations (1.0 g L⁻¹, 1.0 mL L⁻¹, 4.0 mL L⁻¹, and 2.5 mL L⁻¹). The control treatment was distilled water. Chemicals were topically placed in the dorsal region of the tegument of 1 and 3-day old pupae. Then, between 1 and 15-days of emergence of adult parasitoids, residual contact bioassay were used to determine the toxicity of the insecticides. The insecticides thiamethoxam and imidacloprid were harmless to the pupal stage of *E. hispida*, but were harmful to the adult stage. The insecticides deltamethrin and pyriproxyfen were harmful to both pupal and adult stages of *E. hispida*.

Keywords: *Gossypium hirsutum*. Whitefly. Parasitoid. Pesticides.

SELETIVIDADE DE INSETICIDAS A *Encarsia hispida* (Hymenoptera: Aphelinidae)

RESUMO - O uso de inseticidas não seletivos na produção agrícola pode reduzir ou eliminar agentes biológicos que regulam os insetos indesejáveis para o homem no processo produtivo. Neste estudo, o efeito da toxicidade de produtos sintéticos foi avaliado para o parasitoide *Encarsia hispida* tendo como hospedeiro a mosca-branca, *Bemisia tabaci* biótipo B, em algodoeiro. O estudo foi realizado através do uso de bioensaios, usando-se delineamento inteiramente casualizado em esquema fatorial. Nos bioensaios foram utilizadas pupas e adultos de *E. hispida*, as quais foram submetidas à pulverização com os seguintes produtos químicos: tiametoxam, deltametrina, imidacloprido e piriproxifem, nas proporções de 1,0 g L⁻¹, 1,0 mL L⁻¹, 4,0 mL L⁻¹ e 2,5 mL L⁻¹, respectivamente, e um tratamento controle (água destilada). Foram utilizadas pupas com 1 e 3 dias de idade para exposição tópica, mediante aplicação na região dorsal do tegumento. Nos adultos do parasitoide, com idade de 1 e 15 dias de emergência, foram utilizados um bioensaio de contato residual para determinação da toxicidade dos inseticidas. Os inseticidas tiametoxam e imidacloprido são inócuos ao estágio de pupa, no entanto, os mesmos são nocivos à fase adulta de *E. hispida*. Os inseticidas deltametrina e piriproxifem são nocivos para ambas fases pupal e adulta de *E. hispida*.

Palavras-chave: *Gossypium hirsutum*. Mosca-branca. Parasitoide. Agrotóxicos.

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INTRODUCTION

The cultivation of cotton *Gossypium hirsutum* L. var. *latifolium* Hutch is an economically important agribusiness in Brazil, with major technological investments to enhance productive efficiency (OLIVEIRA et al., 2012). However, the insect pests of cotton plants change in response to changing climatic conditions, causing significant losses to agricultural production (LEITE et al., 2010). The behavior and biology of insects have various attributes allowing them to adapt and build resistance to various conditions, including a short life cycle, high fertility, and high capacity of dispersion. One such example of an insect pest on cotton is the whitefly *Bemisia tabaci* Gennadius (PRABHAKER; TOSCANO; HENNEBERRY, 1998; BONATO et al., 2007).

Through applying insecticide products to control of *Bemisia* spp. worldwide, this pest corresponds to a complex of at least 34 morphologically indistinct species (DE BARRO et al., 2011; AHMED et al., 2013). Insecticides also negatively affect the survival of beneficial arthropods. Diamantino et al. (2014) evaluated the selectivity of synthetic products on parasitoids in the cotton plant cultivar DeltaOpal in the southwest of Bahia State, Brazil. The authors found that mortality caused by a variety of products (Fipronil[®] 200 SC, Alfacipermetrina 100 SC, Lufenuron[®] 50 EC, Imidacloprid[®] 200 SC, and Parathion methyl[®] 600 EC) was accentuated in different applications of pesticide. However, following the third application of pesticides, just 26% of the beneficial biological agents survived.

The incorporation of selective insecticides in Integrated Pest Management (IPM) could minimize the deleterious effects of chemical control on non-target organisms (FERREIRA et al., 2006). Among natural enemies, the parasitoid *Encarsia hispida* De Santis has been reported to regulate populations of *B. tabaci* biotype B and the whitefly *Trialeurodes vaporariorum* Westwood naturally on crops of cotton, tobacco, tomato, soybean, cabbage, beans, melons, and eggplant in the midwest region of the Brazilian territory (OLIVEIRA et al., 2003). In the northeast region of Brazil, *E. hispida* was documented parasitizing the whitefly *Trialeurodes variabilis* Quaintance on papaya crops (LOURENÇÃO et al., 2007), with high parasitism potential on *B. tabaci* biotype B hosts in cotton cultivars (OLIVEIRA et al., 2016).

Pedigo and Rice (2009) support the need for control tactics, such as the use of natural enemies and resistant plants, to minimize attacks by pests in IPM. Thus, this study aimed to analyze the toxicity of insecticides to the parasitoid *E. hispida* that has *B. tabaci* biotype B as its host on two cotton cultivars.

MATERIAL AND METHODS

The study was carried out in the Laboratory of Entomology (LEN), Campus II of the Federal University of Paraíba (UFPB), Areia, Paraíba State, Brazil. The experiment was performed under laboratory conditions, at 25 ± 2 °C temperature, $70 \pm 10\%$ relative humidity (R.H.) and a 12 h photo-phase.

Rearing of whitefly and parasitoid

Populations of both the whitefly *B. tabaci* biotype B and the parasitoid *E. hispida* were reared in the LEN, using the methodology proposed by Oliveira et al. (2016) in a greenhouse and laboratory, respectively. We used the cotton cultivars BRS H8 (white) and BRS Topázio (brown) from the National Cotton Research Center of the Brazilian Agricultural Research Corporation (EMBRAPA/CNPA).

The adults of the parasitoids were collected using gelatin capsules 00 Medeiros (2009). After capture, the adults were released next to plants of *Euphorbia pulcherrima* Wild to be colonized containing nymphs of 3rd and 4th instars of *B. tabaci*.

Mortality rate of *E. hispida* pupae

Leaves of both cotton cultivars BRS H8 and BRS Topázio containing pre-pupae of *E. hispida* were collected from the LEN greenhouse and were posteriorly removed using an entomological pin that was placed in Petri dishes (15.0 × 2.0 cm) until they reached the pupal stage. We used 1 and 3-day old parasitoids.

We used a completely randomized design in a factorial scheme (2 × 5) (1 and 3 day-old pupae × five chemical products) for each cotton cultivar, and with four replications. We used the following treatments: (Actara[®] 250 WG), deltamethrin (Decis[®] EC), imidacloprid (Proven[®] 200 SC), and pyriproxyfen (Tiger[®] 100 EC) at concentrations of 1.0 g L⁻¹, 1.0 mL L⁻¹, 4.0 mL L⁻¹, and 2.5 mL L⁻¹. The control treatment used distilled water. The experimental unit consisted of 25 pupae placed in Petri dishes (9.0 × 1.5 cm) containing filter paper. In the pupae, the chemicals were applied topically by manually spraying their entire integument of the pupae. Then, the recipients were arranged in an airy atmosphere and covered with plastic film. The environmental conditions were 26 ± 2 °C, $70 \pm 10\%$ R.H., and 12 h photo-phase.

Five days after the pupae were exposed to the treatments, we recorded the mortality of the parasitoid for the two evaluated ages, viz., days 1 and 3.

Mortality rate of adult *E. hispida*

For the bioassay, the leaves of both cotton cultivars containing pupae of *E. hispida* were collected and transferred to the laboratory. Within 12 h of the parasitoids emerging, they were collected in gelatin capsules 00 (MEDEIROS, 2009) and subjected to the residual contact method proposed by Ozawa, Saito and Ikeda (1998) to determine the mortality rate of the insect. The same insecticides were used for the adult stage of the parasitoid, diluted in acetone (99.5%). Then, an aliquot of 2.5 mL L⁻¹ was placed in a test tube (8.5 × 1.5 cm) so that it spread in the container, and remained in the ventilated environment for 1 h to eliminate the diluent. For the control, we used only the spreader. Thus, the experimental unit consisted of 10 insects placed in containers with honey as food. The test tubes were sealed with a micro-perforated plastic film.

After 24 h of parasitoids being exposed to the insecticides, mortality was recorded using a stereoscopic magnifying glass. The experimental conditions were: 26 ± 2 °C, 70 ± 10% R.H., and a 12 h photo-phase. The experimental design was completely randomized in a factorial scheme (2 × 5) (adult of 1 and 15-days old × five chemical products) for each cultivar, and with four replications.

Statistical Analysis

Mortality (%) was initially corrected for in relation to the mortality recorded in the control, using the formula $Ma = [(Mt-Mc)/(100-Mt)] \times 100$, where Ma = mortality corrected for based on the control treatment; Mt = mortality observed in the

pesticide treatments, and Mc = mortality observed in the control treatment (ABBOTT, 1925). Then, the data were subjected to analysis of variance, and the means were compared by Student-Newman-Keuls test ($p < 0.05$) using Assisat 7.7 program (SILVA; AZEVEDO, 2002).

The insecticides were classified into four categories according to the recommended standards of “International Organization for Biological and Integrated control of Noxious Animals and Plants” (IOBC). Specifically, Class 1 - harmless (<30%); class 2 - slightly harmful (30–79%); class 3 - moderately harmful (80–99%); and class 4 - harmful (>99%) (AMANO; HASEEB, 2001).

RESULTS AND DISCUSSION

The insecticides used in this study clearly affected the pupae of *E. hispida* on both cotton cultivars (Table 1). Thiamethoxam and imidacloprid were harmless (class 1) to the pupal stage in the cultivar BRS H8 for both evaluated periods. In comparison, deltamethrin and pyriproxyfen significantly affected the pupae, and were classified as harmful (class 4) and moderately harmful (class 3) for 1 and 3-day old pupae, respectively. We obtained the same results for the cultivar BRS Topázio. Different deleterious effects in the pupal stage were evidenced because deltamethrin was harmful to the parasitoid. The observed deleterious effects were: 1) lack of viability of pupae, 2) death of adults on emergence, and 3) certain behavioral characteristics, such as tremors and lack of motor coordination in adults after emergence.

Table 1. Mortality (%) of the pupal stage of *Encarsia hispida* (mean* ± SE*) 5 days after insecticides were applied to *Bemisia tabaci* biotype B on two cotton cultivars.

Treatment	Cotton cultivar BRS H8			
	1 day**	C***	3 days**	C***
Thiamethoxam	15.0 ± 3.14 bA	1	3.0 ± 1.96 bcB	1
Deltamethrin	100.0 ± 0.00 aA	4	90.0 ± 3.01 aB	3
Imidacloprid	7.0 ± 2.56 cA	1	8.0 ± 2.72 bA	1
Pyriproxyfen	100.0 ± 0.00 aA	4	94.0 ± 2.38 aB	3
Control	0.0 ± 0.00 dA	-	0.0 ± 0.00 cA	-
Cotton cultivar BRS Topázio				
Thiamethoxam	12.0 ± 3.26 bA	1	18.0 ± 3.14 cA	1
Deltamethrin	100.0 ± 0.00 aA	4	85.0 ± 4.76 bB	3
Imidacloprid	9.0 ± 2.87 bA	1	4.0 ± 1.96 dA	1
Pyriproxyfen	100.0 ± 0.00 aA	4	98.0 ± 1.40 aA	3
Control	0.0 ± 0.00 cA	-	0.0 ± 0.00 dA	-

* = Averages followed by the same lowercase letter in columns and uppercase letter in rows did not differ when using the Student-Newman-Keuls test ($p < 0.05$); ** = Day (s) - age of the pupae; *** = Class 1 - harmless (<30%), Class 2 - slightly harmful (30–79%), Class 3 - moderately harmful (80–99%), Class 4 - harmful (>99%).

The presence of residues of deltamethrin in the tegument of the parasitoid pupae caused the adults to die on emergence, possibly harming the pupae too. This product clearly affected the nervous

system of the insect. According to Spencer et al. (2001), insecticides interfere with the opening and closing of neural sodium channels, extending the time that Na⁺ enter, which results in the transmission

of repetitive and uncontrolled impulses, hyperexcitability, loss of locomotor posture (“*Knockdown*”), and, probably, paralysis and death. This insecticide was also statistically different in the analyzed periods for both cultivars with 3-day old individuals; however, the mechanisms were not known.

The insecticides deltamethrin and pyriproxyfen were highly toxic before parasitoids emerged for both cotton cultivars. However, there were survivors when these insecticides were applied on 3-day old pupae. The compounds of juvenile hormones, or similar hormones, might act through different connections between cells and proteins (DHADIALLA; CARLSON; LE, 1998). Thus, the mortality of pupae by the insecticide pyriproxyfen might be linked to its mode of action. This mode of action might affect the hormonal balance of insects when applied, interfering with the metamorphosis process, inhibiting juvenile hormone, resulting in the insect not being viable, leading to death.

We found that pyriproxyfen had no statistically different effect on 1 and 3-day old pupae in the cultivar BRS H8. The non-suppression of individuals (pupae) at these ages might be related to their tegument being more developed, preventing the insecticide from penetrating the insect.

Thiamethoxam and imidacloprid might have been harmless to the pupae of the parasitoid because the compounds could not penetrate the exuviae of the host (whitefly), which served as a mechanical barrier (i.e., the parasitoid had no direct contact with these products). The parasitoid did not emerge from its cocoon in one case, possibly because of the effects of insecticide residues when the parasitoid opened the cocoon during emergence, leading to death. These effects occurred because the insecticides penetrated structures called blowholes at the time of emergence and/or orally when the cocoon was opened during emergence. Acetylcholine is the main neurotransmitter in the central nervous system (CNS) of insects. Its action is modulated by the enzyme acetylcholinesterase (AChE), which interrupts the transmission of nerve impulses through the hydrolysis of acetylcholine in acetate and choline (NAUEN et al., 2001).

The results of the present study regarding thiamethoxam differed to those of Vieira et al. (2012). The authors tested the selectivity of insecticides on 7-day old pupae of the parasitoid *Encarsia formosa* Gahan, showing that the chemicals

thiamethoxam 25 and thiamethoxam 50 (neonicotinoid chemical group) caused significant mortality to the parasitoid. As a result, they classified these two chemicals as slightly harmful (class 2) and moderately harmful (class 3), respectively. The authors suggested that the insecticide piriproxifem was moderately harmful to the pupal stage of the biological agent, which was supported by the results of the present study when using 3-day old pupae.

A previous study showed that 0.05 mL L⁻¹ and 1.0 mL L⁻¹ concentrations of piriproxifem reduced the emergence of *E. formosa* pupae by 44.6% and 73.5% (LIU; STANSLY, 1997). This result contrasted with the current study, in which this compound eliminated from 94% to 100% of *E. hispida* pupae. Liu and Stansly (1997) observed that 40% of adults that emerged from pupae had deformed wings, affecting flight, when 1.0 g L⁻¹ of the compound was used. The parasitoids showed typical behavior in relation to the location of the host habitat, host selection, host acceptance, host adequacy, and oviposition.

With respect to imidacloprid, the results of the current study on *E. hispida* pupae contrast with those of Sugiyama, Katayama and Saito et al. (2011). The authors classified this compound as slightly harmful to both the pupae and adults of *E. formosa*. We verified that the product was applied to the pupal stage of *E. hispida* at both ages according to the International Organization for Biological and Integrated control of Noxious Animals and Plants classification, thus making it possible to use it to regulate the *B. tabaci* biotype B.

All insecticides were classified as harmful to *E. hispida* adults reared on both cotton cultivars, except for the 15-day-old adults exposed to pyriproxyfen on both cultivars (Table 2). We verified that the insecticides from different chemical groups (i.e., neonicotinoids, pyrethroids, and pyridyl oxy ether) affect the parasitoid, regardless of the mode of action of the chemical. The insecticides from two of these groups (neonicotinoids and pyrethroids) eliminated the parasitoids in less than 2 h after exposure to the environment with chemical residues. Therefore, the insecticides probably penetrated the body of the insect, depending on the thickness and chemical composition of the cuticle. In general, lipophilic compounds usually penetrate the insect body at higher rates (LEITE et al., 1998).

Table 2. Mortality (%) of the adult stage of *Encarsia hispida* (mean* ± SE*) one day after insecticides were applied to the host, *Bemisia tabaci* biotype B, on two cotton cultivars.

Treatment	Cotton cultivar BRS H8			
	1 day**	C***	15 days**	C***
Thiamethoxam	100.0 ± 0.00 aA	4	100.0 ± 0.00 aA	4
Deltamethrin	100.0 ± 0.00 aA	4	100.0 ± 0.00 aA	4
Imidacloprid	100.0 ± 0.00 aA	4	100.0 ± 0.00 aA	4
Pyriproxyfen	100.0 ± 0.00 aA	4	91.6 ± 3.59 bB	3
Control	0.0 ± 0.00 bA	-	0.0 ± 0.00 cA	-
Cotton cultivar BRS Topázio				
Thiamethoxam	100.0 ± 0.00 aA	4	100.0 ± 0.00 aA	4
Deltamethrin	100.0 ± 0.00 aA	4	100.0 ± 0.00 aA	4
Imidacloprid	100.0 ± 0.00 aA	4	100.0 ± 0.00 aA	4
Pyriproxyfen	100.0 ± 0.00 aA	4	93.3 ± 0.03 bB	3
Control	0.0 ± 0.00 bA	-	0.0 ± 0.00 cA	-

* = Averages followed by the same lowercase letter in columns and uppercase letter in rows did not differ when using the Student-Newman-Keuls test ($p < 0.05$); ** = Day (s) - age of the pupae; *** = Class 1 - harmless (<30%), Class 2 - slightly harmful (30-79%), Class 3 - moderately harmful (80-99%), Class 4 - harmful (>99%).

CONCLUSIONS

The insecticides thiamethoxam and imidacloprid are harmless to the pupal stage of *E. hispida* but are harmful to the adult stage.

The insecticides deltamethrin and pyriproxyfen are harmful to both the pupal and adult stages of *E. hispida*.

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