

SUSCEPTIBILITY OF *Podisus nigrispinus* (DALLAS) (HETEROPTERA: PENTATOMIDEA) TO GAMMA-CYHALOTHRIN UNDER LABORATORY CONDITIONS

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ABSTRACT: *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) is an important biological control agent of the cotton leafworm [*Alabama argillacea* (Hübner)] in the cotton crops in Brazil. The use of selective products such as pyrethroids has been emphasized in this agroecosystem. Then, the susceptibility of the predatory stinkbug *P. nigrispinus* to the pyrethroid insecticide gamma-cyhalothrin was studied in the laboratory. Nymphs and adults of the predator were exposed to seven concentrations of the gamma-cyhalothrin via topical contact and ingestion through treated water. The concentrations studied of this insecticide via topical application were relatively harmless for adults of *P. nigrispinus* (females: $LC_{50} = 143.5$ ppm a.i., and males: $LC_{50} = 145.1$ ppm a.i.) and 5th-instar nymphs ($LC_{50} = 103.9$ ppm) as compared to via ingestion (females: $LC_{50} = 1.98$ ppm a.i., males: $LC_{50} = 1.91$ ppm a.i., and 5th-instar nymphs: $LC_{50} = 2.05$ ppm a.i.). The use of pyrethroids and beneficial insects in IPM programs should be better evaluated.

Key words: cotton, biological control, pyrethroid, selectivity, natural enemies

SUSCETIBILIDADE DE *Podisus nigrispinus* (DALLAS) (HETEROPTERA: PENTATOMIDAE) A GAMMA-CYHALOTHRIN SOB CONDIÇÕES DE LABORATÓRIO

RESUMO: *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) é um importante agente de controle biológico do curuquerê [*Alabama argillacea* (Hübner)] na cultura do algodão no Brasil. O uso de produtos seletivos tais como piretróides tem sido enfatizado no agroecossistema do algodoeiro. Portanto, estudou-se em laboratório a suscetibilidade do percevejo predador *Podisus nigrispinus* (Dallas) ao inseticida piretróide gamma-cyhalothrin. As exposições via ingestão e aplicação tópica de ninfas de 5º instar, fêmeas e machos de *P. nigrispinus* a gamma-cyhalothrin foram feitas, usando-se sete concentrações do inseticida. Gamma-cyhalothrin via aplicação tópica foi relativamente menos tóxico às formas adultas de *P. nigrispinus* (fêmeas: $CL_{50} = 143,5$ ppm i.a. e machos: $CL_{50} = 145,1$ ppm i.a.) e às ninfas de 5º instar ($CL_{50} = 103,9$ ppm) desse predador, quando comparado a sua utilização por ingestão (fêmeas: $LC_{50} = 1,98$ ppm i.a., machos: $LC_{50} = 1,91$ ppm i.a. e ninfas de para ninfas de 5º instar: $LC_{50} = 2,05$ ppm i.a.). Os resultados dessa pesquisa sugerem que o uso de piretróides em programas de MIP deve ser avaliado com bastante cautela.

Palavras-chave: algodão, controle biológico, piretróide, seletividade, inimigo natural

INTRODUCTION

Integrated Pest Management in cotton is based mainly on the action of predators (Whitcomb & Bell, 1962; Ramalho, 1994), and parasitoids (Ruberson et al., 1994; Ramalho et al., 2000) to suppress pest populations. Insecticides are used only when pest populations threaten to exceed the economic threshold. Investigations on side-effects of insecticides and other pesticides play a crucial role in IPM programs, when chemical and biological measures are used in combination against pests (Mohaghegh et al., 2000, Zanuncio et al., 2003). Then, the insecticides should be effective against insect pests, but relatively safe

to natural enemies, and that requires knowing the complex of natural enemies affecting key pests species and the impact of insecticides on these organisms (Campbell et al., 1991).

Biological control has many advantages over other control methods because it is safe, permanent, and economic when correctly used (Stehr, 1982). However, natural enemies may be affected by insecticide sprays via contact with residues, or indirectly through contaminated food or water (Mestdagh et al., 1996). Behavioral and pesticide processes may contribute to differences on susceptibility of predaceous insects and their preys to insecticides (Boyd & Boethel, 1998).

Podisus nigrispinus (Dallas) (Heteroptera: Pentatomidae) is an important natural enemy of cotton crop pests in Brazil (Medeiros et al., 2000; 2003). Because of poisoning risks, the use of selective products such as pyrethroids has been emphasized in this agroecosystem (Tillman & Mulrooney, 2000).

Smagghe & Degheele (1995) studying the effects of RH 5849 and RH 5992 (tebufenozide) on *P. nigrispinus*, concluded that their nymphs did not show sensitivity to oral treatment (feeding on treated prey) or topical exposure. According to Picanço et al. (1997), permethrin, followed by deltamethrin and methil parathion were the most selective insecticides to *P. nigrispinus* by contact exposure, in comparison to other groups of insecticides. The selectivity of chemical products to *P. nigrispinus* have been studied; however, few researches analyzed side-effects of pyrethroids on this predator. In this work the susceptibility of *P. nigrispinus* to the new pyrethroid insecticide gamma-cyhalothrin was studied in the laboratory.

MATERIAL AND METHODS

Insects - Newly molted 5th-instar nymphs (≤ 24 h old), and male and female adults (≤ 24 h old) of *Podisus nigrispinus* were obtained from the mass rearing facility of the Biological Control Unit (BCU)/Embrapa Algodão, Campina Grande, State of Paraíba, Brazil. The insects were maintained in growth chambers at 26°C, 60 \pm 10% relative humidity, and 12L:12D photoperiod. The prey used to feed this predator was larvae of *Musca domestica* L. (Diptera: Muscidae) originated from colonies at BCU.

Chemical - Gamma-cyhalothrin 15% suspension concentrate (SC) ((S)-a-cyano-3-phenoxybenzyl(Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate) was supplied by Dow AgroSciences Industrial Ltda, Brazil. Gamma-cyhalothrin is the isolated active isomer of lambda-cyhalothrin.

Topical Toxicity - Seven concentrations of the gamma-cyhalothrin (11.7, 23.4, 46.9, 93.8, 187.5, 375.0, and 750.0 ppm a.i for 5th-instar nymphs, and 23.4, 46.9, 93.8, 187.5, 375.0, 750.0, and 1.500 ppm a.i for adult females and males) were topically applied on individuals of *P. nigrispinus*. One microliter of each concentration of this insecticide diluted in water was applied using a micro syringe on the thoracic dorsum of newly molted 5th-instar and adults of this predator; 1 μ L of water was used as control. Insects were individually placed in small plastic cups (7.0 cm height; 10 cm diameter) with a hole in their cover. A total of 224 females, 230 males, and 250 5th-instar nymphs were used in the topical toxicity assay. A 2.5 μ L plastic tube with distilled water and plugged with cotton was inserted in its hole. To supply water to the predator and to maintain the humidity inside the cups. In-

dividuals of *P. nigrispinus* received *M. domestica* larvae immediately after treated with the gamma-cyhalothrin. Nymphs of this predator were checked for mortality every 24 h until eight days after molting and survival of adults was monitored up to eight days after emergence. Mortality included dead individuals and those without movements.

Oral Toxicity - Individuals of *P. nigrispinus* were exposed to the following concentrations of gamma-cyhalothrin: 0.47, 0.94, 1.88, 3.75, 7.50, 15.0, and 30.0 ppm a.i by ingestion through treated drinking water. The insecticide solution was offered to the predators through 2.5 mL cylindrical plastic tubes, inserted in the cover of each cup of 7.0 cm height and 10 cm diameter. Two hundred and forty five, 5th-instar nymphs, 280 males and 270 females were used in the oral toxicity study. Control groups received distilled water. During the first 24 hours, no food was supplied to stimulate the drinking behavior of *P. nigrispinus*.

P. nigrispinus were fed with *M. domestica* larvae just after treated with the gamma-cyhalothrin. Mortality of nymphs of this predator was evaluated up to eight days and its adults until eight days old. Mortality included dead individuals and those without movements. Only the mortality data at eight days post-application of the insecticide were submitted to probit analysis.

Statistical Analysis - Data were analyzed by probit analysis (Finney, 1971) using probit procedures (SAS Institute Inc., 2003), and significance was assessed by the degree of overlap of 95% CL, i.e., no overlap indicates a significant difference at $P \leq 0.05$.

RESULTS

P. nigrispinus was affected by the gamma-cyhalothrin (Figures 1-3, Table 1). The mortality of *P. nigrispinus* over the time increased at different percentages (Figures 1-3). Final percentages of mortality for 5th-instar nymphs (at 750 ppm a.i) (Figure 1), female adults (1.500 ppm a.i) (Figure 2), and male adults (1.500 ppm a.i) (Figure 3) of *P. nigrispinus*, exposed to gamma-cyhalothrin by topical application were 96.5%, 90.0%, and 90.1%, respectively. The percentages of mortality for 5th-instar nymphs (at 30 ppm a.i), female adults (30 ppm a.i), and male adults (30 ppm a.i) of *P. nigrispinus* exposed to gamma-cyhalothrin by ingestion at 8 days post-application were 98.8%, 100.0%, and 100.0%, respectively (Figures 1-3). Values of the LC_{50} of gamma-cyhalothrin via topical application were 103.9, 143.6, and 145.2 ppm a.i for 5th-instar nymphs, female and male adults, respectively (Table 1). On the other hand, these values were 2.0, 2.0 and 1.9 ppm a.i for 5th-instar nymphs and female and male adults of *P. nigrispinus*, respectively after exposure to the gamma-cyhalothrin via ingestion (Table 1).

Table 1 - Toxicity of the gamma cyhalothrin to 5th-instar nymphs and adult females and males of *Podisus nigrispinus* (Heteroptera: Pentatomidae) via topical exposure and ingestion at 26°C, relative humidity of 60 ± 10%, and a photoperiod of 12L:12D.

| Application method | Stage | n | Slope ± SE | LC ₁₀ (95% FL) | LC ₅₀ (95% FL) | LC ₉₀ (95% FL) | χ ² ; P > 0 | df |
|--------------------|------------------|-----|-------------|---------------------------|---------------------------|--------------------------------|------------------------|----|
| Topical exposure | 5th-instar nymph | 250 | 1.62 ± 0.12 | 16.74 (11.73-30.35) a | 103.94 (87.34-123.75) a | 645.35 (486.83-924.07) a | 186.81; 0.0001 | 1 |
| | Adult female | 224 | 1.15 ± 0.13 | 11.12 (3.37-22.37) a | 143.56 (95.33-207.80) a | 1,854.00 (1,004.00-5,183.00) b | 81.05; 0.0001 | 1 |
| | Adult male | 230 | 1.13 ± 0.12 | 10.68 (3.26-21.52) a | 145.16 (96.99-209.18) a | 1,972 (1,065-5,502) b | 88.54; 0.0001 | 1 |
| Ingestion | 5th-instar nymph | 245 | 1.28 ± 0.17 | 0.21 (0.05-0.45) b | 2.05 (1.21-3.12) b | 20.32 (11.03-61.69) c | 69.37; 0.0001 | 1 |
| | Adult female | 270 | 1.32 ± 0.14 | 0.21 (0.07-0.41) b | 1.98 (1.31-2.80) b | 18.37 (11.06-41.65) c | 84.57; 0.0001 | 1 |
| | Adult male | 280 | 1.94 ± 0.13 | 0.42 (0.30-0.53) b | 1.91 (1.63-2.21) b | 8.73 (7.08-11.29) c | 209.34; 0.0001 | 1 |

n = number of insects treated.

The value of each χ² refers the probability of the angular coefficient > 0.

LC - values and slopes (in ppm a.i) were estimated by probit procedure (SAS Institute Inc., 2003).

Values followed by the same letter within row are not significantly different if their 95% CL overlap.

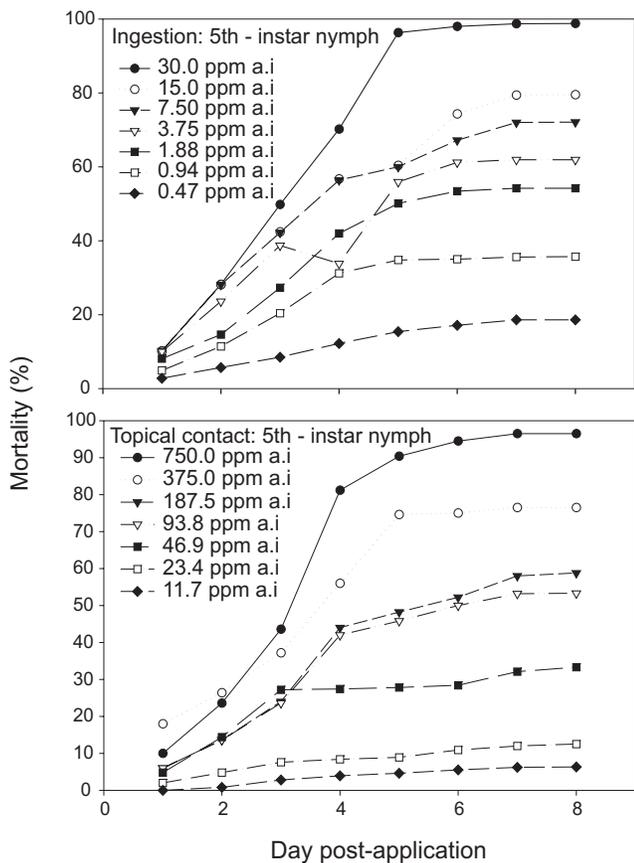


Figure 1 - Average mortality of 5th-instar nymphs of *P. nigrispinus* treated topically (n = 250) and by ingestion (n = 245) with gamma-cyhalothrin.

This insecticide via topical application was relatively harmless for adults of *P. nigrispinus* (females: LC₅₀ = 143.5 ppm a.i. and males: LC₅₀ = 145.1 ppm a.i.), and 5th-instar nymphs (LC₅₀ = 103.9 ppm) in comparison to via ingestion (females: LC₅₀ = 1.98 ppm a.i., males: LC₅₀ = 1.91

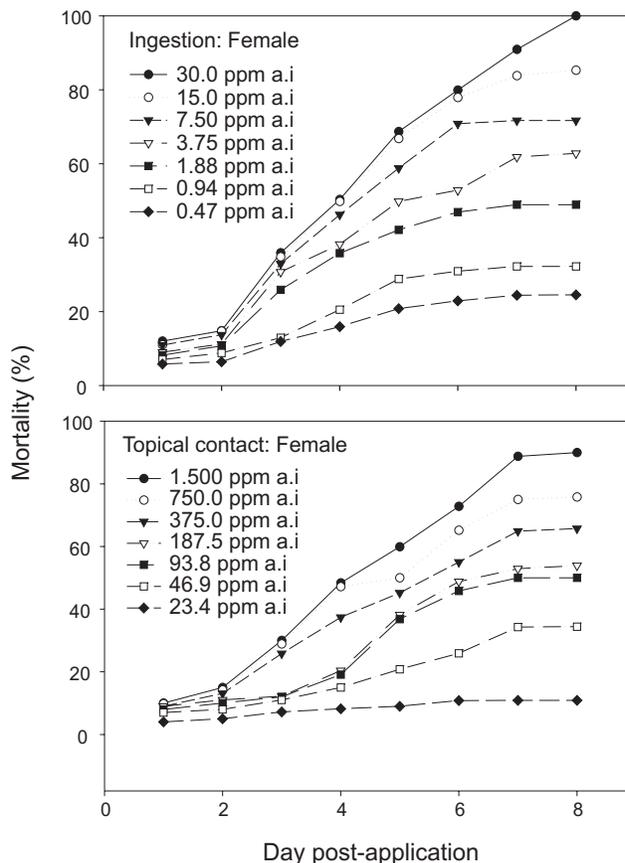


Figure 2 - Average mortality of females of *P. nigrispinus* treated topically (n = 224) and by ingestion (n = 270) with gamma-cyhalothrin.

ppm a.i., and 5th-instar nymphs: LC₅₀ = 2.05 ppm a.i.). Therefore, *P. nigrispinus* was more affected by drinking water treated with gamma-cyhalothrin (average LC_{50's} = 1.98 ppm between nymphs and adults) than by topical exposure (average LC_{50's} = 130.88 ppm between nymphs and adults).

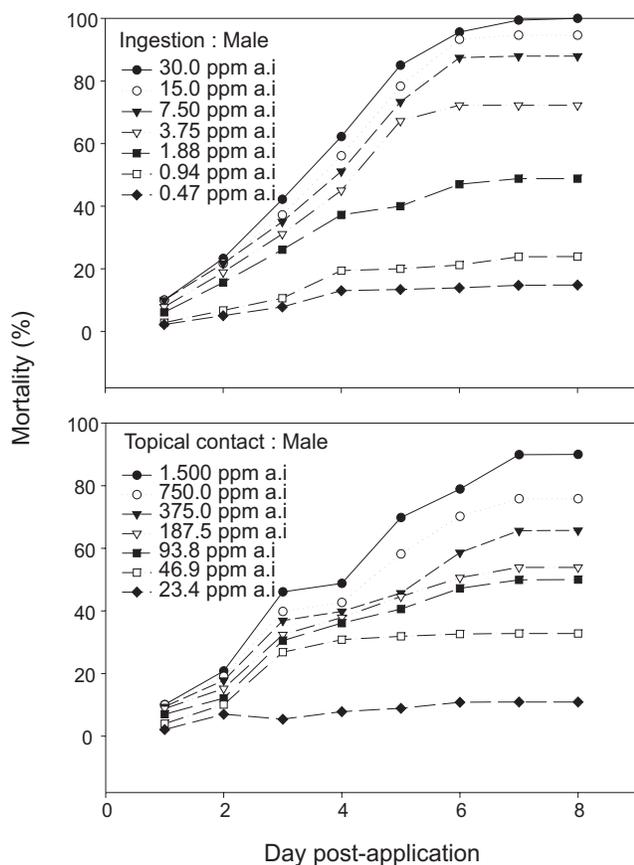


Figure 3 - Average mortality of males of *P. nigrispinus* treated topically (n = 230) and by ingestion (n = 280) with gamma-cyhalothrin.

DISCUSSION

Predatory stinkbugs may be affected by insecticide applications via direct and residual contact, or through feeding on contaminated prey or plants. On the other hand, plant feeding has positive implications on predaceous bugs for biological control. Plant material can be an alternative food to maintain populations of omnivorous predators when preys are scarce (Ehler & Miller, 1978). Phytophagy helps to maintain high population levels of *P. nigrispinus* (Lemos et al., 2001) because predatory Pentatomidae can obtain moisture and maybe nutrients from plants (De Clercq & Degheele, 1992). Consequently, these predators may be poisoned by contaminated water or juices from insecticide-treated plants (De Clercq et al., 1995).

Researchers have studied the tolerance of *Podisus* spp. to insecticides. *Podisus maculiventris* (Say) nymphs and adults showed high mortality after exposed by tarsal contact to organophosphate insecticides (Wilkinson et al., 1978). *P. maculiventris* is more susceptible to organophosphate insecticides sulprofos and profenofos than to pyrethroids fenvalerate and permethrin (Wilkinson et al., 1979). This predator is generally more susceptible to or-

ganophosphate and carbamate insecticides than to pyrethroids compared to its prey (Yu, 1988). The pyrethroid insecticides fenvalerate and deltamethrin are less toxic to *P. nigrispinus* than the organophosphate fenitrothion (Guedes et al., 1992). The susceptibility of insects to insecticides will depend on the ability of an insect to handle these compounds and the avoidance of direct contamination or deleterious effects in successive generations can determine the degree of insect resistance.

The pyrethroid gamma-cyhalothrin was more toxic to *P. nigrispinus* by ingestion than by topical application. This observation corroborates of De Cock et al. (1996), who reported a high toxicity of imidacloprid insecticide to fifth instar nymphs and adults of *P. maculiventris* by ingestion as compared to topical exposure. De Clercq et al. (1995) found higher mortality caused by diflubenzuron in the drinking water to *P. maculiventris* than pyriproxyfen; however diflubenzuron was not active by residual and direct contacts. Benzoylphenyl ureas are more toxic to *P. nigrispinus* by ingestion than by contact or topical application because its absorption through the cuticle is relatively low, and that may explain its selectivity towards beneficial insects (Retnakaran & Wright, 1987). The exact reason of lower susceptibility of natural enemies such as *Podisus* spp. to pyrethroids is not well known (Picanço et al., 1997). The hypothesis of a slower penetration of these insecticides through the cuticle of *P. nigrispinus* are discussed because this insect has thicker, lipid-rich cuticle than its prey (Guedes et al., 1992). Therefore, this tolerance may increase the efficiency of *P. nigrispinus* in biological control programs.

Nymphs of *P. maculiventris* were more susceptible after topical exposure to imidacloprid than adults (De Cock et al., 1996) what was also found for *P. nigrispinus* after topical exposure to gamma-cyhalothrin. This may explain the different cuticular penetration rate of insecticides topically applied to *P. maculiventris* (Yu, 1988). Also, an excess of cuticle may deposit highly lipophilic insecticides, what depends on food intake and its effects on composition of cuticles, especially on lipid content (Ebeling, 1964). The rate of penetration of lipophilic insecticides in the cuticle is slower in adult predators due to their thicker and more rigid cuticle than their nymphs. This reduce the acquisition process and may explain differences on the toxicity of the gamma-cyhalothrin to this predator. Higher toxicity of this insecticide to *P. nigrispinus* via ingestion may be due to the fact that this insecticide enters the circulation and contaminate hemolymph supply. This increases its distribution through the organism without any efficient natural barrier such as the cuticle.

The most common type of resistance is the removal of the insecticide from its site of action by detoxication (Perry & Agostin, 1974). The biotransformation

(substances able to neutralize, inactivate, or eliminate foreign organic substances, including poisons or to avoid toxic damage) may involve oxidation, reduction, hydrolytic processes, and synthetic or conjugative reactions (Williams, 1959). Yu (1987) reported higher amount of the detoxication enzymes (microsomal oxidases and esterases) in predatory stinkbugs than in its prey what indicates mechanism of tolerance to insecticides in pentatomids. The selectivity of gamma cyhalothrin to *P. nigrispinus* may be related to the two protection mechanisms herein discussed. However, the combined use of pyrethroids and beneficial insects in IPM programs should be better evaluated.

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