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MONITORING THE RESISTANCE OF TRIATOMINAE (HEMIPTERA: REDUVIIDAE) TO INSECTICIDES

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Abstract Currently, there is evidence of *Triatoma infestans* Klug, 1834 populations from southern Bolivia and northern Argentina with high levels of resistance to pyrethroids. In this study we characterized the susceptibility levels of 37 Triatominae populations to deltamethrin. The bioassays were performed by topical application of insecticides in the abdomen of 1st stage nymphs of *Triatoma brasiliensis* Neiva, 1911 and *Triatoma sordida* Stål, 1859 coming from the Northeast and Midwest regions of Brazil. Estimates of LD_{50} and RR_{50} of the different field populations showed high levels of susceptibility (LD_{50} <1 and RR_{50} <3) for all samples. It is suggested that the high spatial mobility of *T. brasiliensis* can be considered as the main obstacle to the selection of resistant individuals. The tests conducted showed that there is no need to replace the insecticide used in campaigns for vector control of Chagas disease in Brazil. However, temporal changes of susceptibility should be continuously monitored as well as the resistance mechanisms and their evolution in order to properly guide the actions of vector control.
Key Words Deltamethrin, *Triatoma brasiliensis*, *Triatoma sordida*,Topical bioassays.

INTRODUCTION

Currently, *Triatoma brasiliensis* Neiva, 1911 is the most important vector of Chagas Disease (CD) throughout Northeastern Brazil. Epidemiological studies conducted in the states of Bahia (Walter et al., 2005), Ceará (Sarquis et al., 2004) and Piauí (Soares et al.,2000) showed that *T. brasiliensis* is the most frequent species in the domestic environment with high levels of natural infection with *Trypanosoma cruzi* Chagas, 1909, becoming one of the most efficient transmitters of CD to humans.

Also noteworthy is *Triatoma sordida* Stål 1859, which is the most frequently captured triatomine species in Brazil, mainly in peridomestic environment. In this environment, *T. sordida* specimens can be found inhabiting biotopes, such as wood and tile piles, wooden fences, chicken coops and pig pens (Diotaiuti et al. 1998; Forattini et al., 1971).

The reduction and / or elimination of vector populations inside and outside homes has been the basic strategy of controlling the transmission of CD, which is performed by means of chemical treatment with residual insecticide spraying. In Brazil, originally, the triatomine control actions were carried out with Benzenehexachoride (BHC) – an organochlorine insecticide. However, since 1983, the National Program for Chagas Disease Control (NPCDC) has instituted the use of synthetic pyrethroids (Silveira, 2004).

Despite the continuous and intensive control campaigns against the vectors of CD, few studies have been performed to detect changes in susceptibility of triatomine populations in relation to insecticides used for their control. The objective of this study was to examine the levels of susceptibility of *T. brasiliensis* and *T. sordida* to deltamethrin in order to subsidize a monitoring program for the resistance of DC vectors, which are essential not only for Brazil but also throughout Latin America.

MATERIAL AND METHODS

The reference populations of *T. brasiliensis* and *T. sordida* came from laboratory-reared colonies since February, 1984 and March, 1982, respectively. The criteria for selecting the reference population and to monitor bug resistance are established in the protocol of the World Health Organization (WHO, 1994). Field insects were obtained from endemic areas treated with insecticides, in which Chagas disease control programs perform continuous and systematic applications with residual insecticide spraying. The insecticide used was Deltamethrin [(S) - ά - cyano - 3 - phenoxybenzyl (1R) - cis - 3 - (2,2 - dibromovinyl) - 2,2 dimethyl cyclopropane carboxylate] with a purity of 98.2% granted by Bayer ® (Brazil). At least five concentrations of the insecticide deltamethrin were used to estimate mortality rates from 0 to 99%. The biological tests consisted of topical application of a deltamethrin solution (0.2 µl) in the abdomen of fasting 1^{st} instar nymphs with five to seven days of age. The same volume of acetone was administered in the insects of the control group. All tests were repeated in the laboratory, at least five times, on different days. The mortality reading was accomplished after 24 hours exposure of the insecticide and the lethal doses were expressed in nanograms per insect (ng/insect). The LD_{50} and LD_{95} calculation of each population was based on the Probit analysis. Estimates of the LD_{50} and LD_{95} variation among populations were compared by analysis of variance (ANOVA) and Tukey tests. The values of RR_{50} and RR_{95} were considered significantly different when there was no overlap of the limits of the confidence intervals at 95% of the reference population with the field samples.

RESULTS AND DISCUSSION

The estimated values of susceptibility of *T. brasiliensis* reference strain to deltamethrin were 0.68 ng / insect (LD_{50}) and 1.58 ng/insect (LD_{95}) (Table 1). The LD_{50} values of the different field populations ranged from 0.68 ng / insect to 1.48 ng / insect. The statistical analysis indicated a significant difference between LD_{50} (ANOVA $F_{16, 74}$= 18.1, p<0.01) and LD_{95} (ANOVA $F_{16, 74}$= 19.9, p<0.01) of the studied populations.

Considering *T. sordida,* the estimates of LD_{50} and LD_{95} for the reference strain were 0.58 ng / insect and 1.35 ng / insect, respectively. The susceptibility of triatomines to deltamethrin was evaluated in 21 *T. sordida* populations from different municipalities in the Midwest region of Brazil. The LD_{50} and LD_{95} variation is shown in Table 2. The analysis of variance has detected a significant difference of LD_{50} (ANOVA $F_{21, 92}$= 30.4, p<0.01) and LD_{95} (ANOVA $F_{21, 92}$= 15.5, p<0.01) among *T. sordida* populations.

Table 1. Susceptibility of *Triatoma brasiliensis* populations to deltamethrin. The number of specimens tested, slope, lethal doses (LD_{50} and LD_{95}) and resistance rations (RR_{50} and RR_{95}) are shown for each population. TbREF: Umari/CE. TbPBItV: Itaporanga/PB. TbPBMAgC: Mãe d' Água/PB. TbPBMoO: Monteiro/PB. TbPBPiJ: Piancó/PB. TbPBSCrG: Santa Cruz/PB. TbPBSFrS: São Francisco/PB. TbPBSJEsL: São José de Espinharas/PB. TbPELGrM: Lagoa Grande/PE. TbPEPeL: Petrolina/PE. TbPESaS: Salgueiro/PE. TbPESTaJ: Serra Talhada/PE. TbPIOeP, TbPIOeT, TbPIOeF e TbPIOeS: Oeiras/PI. TbRNCaP: Caicó/RN.

Populations	N	Slope ± SD	LD_{50} ng/insect (95% IC)	LD_{95} ng/insect (95% IC)	RR_{50} (95% CI)	RR_{95} (95% CI)
TbREF	910	4,51 ± 0,37	0,68 (0,64 - 0,73)	1,58 (1,37 - 1,91)	1,00 (0,91 - 1,09)	1,00 (0,79 - 1,26)
TbPBItV	770	3,52 ± 0,47	0,68 (0,57 - 0,78)	2,01 (1,72 - 2,59)	1,00 (0,85 - 1,18)	1,27 (0,99 - 1,63)
TbPBSJEsL	700	4,74 ± 0.46	0.73 (0,67 - 0,78)	1,62 (1,42 - 1,94)	1,06 (0,96 - 1,17)	1,02 (0,82 - 1,27)
TbPBSFrS	700	4,83 ± 0,46	0,74 (0,69 - 0,79)	1,63 (1,44 - 1,95)	1,09 (0,99 - 1,20)	1,03 (0,82 - 1,28)
TbPESaS	700	3,24 ± 0,26	0,77 (0,69 - 0,84)	2,49 (2,14 - 3,04)	1,13 (1,00 - 1,27)	1,57 (1,23 - 1,99)
TbPEPeL	900	3,21 ± 0,26	0,79 (0,71 - 0,86)	2,58 (2,22 - 3,16)	1,16 (1,03 - 1,30)	1,63 (1,28 - 2,07)
TbPBPiJ	400	2,96 ± 0.38	0,81 (0,69 - 0,93)	2,94 (2,28 - 4,39)	1,19 (1,01 - 1,40)	1,85 (1,30 - 2,63)
TbPBMoO	700	4,59 ± 0,43	0,82 (0,76 - 0,88)	1,88 (1,62 - 2,30)	1,20 (1,09 - 1,32)	1,18 (0,93 - 1,49)
TbPBMAgC	700	3,29 ± 0,31	0,83 (0,75 - 0,90)	2,62 (2,20 - 3,36)	1,21 (1,08 - 1,36)	1,65 (1,27 - 2,16)
TbRNCaP	800	3,17 ± 0.23	0,85 (0.76 - 0.93)	2,80 (2,44 - 3,34)	1,24 (1,09 - 1,40)	1,77 (1,41 - 2,22)
TbPESTaJ	900	3,39 ± 0,38	0,85 (0,77 - 0,95)	2,61 (2,06 - 3,75)	1,25 (1,11 - 1,41)	1,65 (1,19 - 2,28)
TbPBSCrG	700	3,49 ± 0,26	0,97 (0,89 - 1,04)	2,86 (2,48 - 3,45)	1,41 (1,27 - 1,57)	1,80 (1,43 - 2,28)
TbPELGrM	800	3,36 ± 0,32	1,10 (0,99 - 1,22)	3,41 (2,85 - 4,39)	1,61 (1,43 - 1,82)	2,15 (1,64 - 2,81)
TbPIOeP	900	2,82 ± 0,20	1,12 (1,01 - 1,23)	4,30 (3,61 - 5,40)	1,66 (1,47 - 1,87)	2,85 (2,19 - 3,71)
TbPIOeT	900	2,83 ± 0,21	1,20 (1,08 - 1,32)	4,56 (3,84 - 5,71)	1,75 (1,55 - 1,98)	2,88 (2,23 - 3,72)
TbPIOeF	900	2,74 ± 0,19	1,33 (1,20 - 1,46)	5,29 (4,38 - 6,74)	1,94 (1,73 - 2,19)	3,43 (2,55 - 4,38)
TbPIOeS	1100	3,70 ± 0,29	1,48 (1,38 - 1,61)	4,13 (3,51 - 5,14)	2,17 (1,96 - 2,40)	2,61 (2,03 - 3,35)

Table 2. Susceptibility of *Triatoma sordida* populations to deltamethrin. The number of specimens tested, slope, lethal doses (LD_{50} and LD_{95}) and resistance rations (RR_{50} and RR_{95}) are shown for each population. TsREF: Cordeiros/BA. TsBACafB: Cafarnaum/BA. TsBACarM: Carinhanha/BA. TsBAMaS: Malhada/BA. TsBAMuB: Mucugê/BA. TsBANReA: Nova Redenção/BA. TsBAPaR: Palmeiras/BA. TsBARCoB: Rio de Contas/BA. TsBAXXiR: Xique-Xique/BA. TsGOFiC: Firminópolis/GO. TsGOGGoS: Guarani do Goiás/GO. TsGOPoE: Posse/GO. TsGOSLMBeE: São Luis Montes Belos/GO. TsMTPoM: Poxoréo/MT. TsMTSJPoS: São José do Povo/MT. TsMSCGrA: Campo Grande/MS. TsMSATaJ: Aparecida do Taboado/MS. TsMSDoM: Douradina/MS. TsMSRoE: Rochedo/MS. TsMSTeC: Terenos/MS. TsMGLoR: Lontra/MG. TsTOAToM: Aurora do Tocantins/TO.

Populations	N	Slope ± SD	LD_{50} ng/insect (95% IC)	LD_{95} ng/insect (95% IC)	RR_{50} (95% CI)	RR_{95} (95% CI)
TsREF	800	4,51 ± 0,34	0,58 (0,54 - 0,63)	1,35 (1,20 - 1,57)	1,00 (0,90 - 1,10)	1,00 (0,82 - 1,20)
TsBAXXiR	800	4,52 ± 0,34	0,61 (0,56 - 0,66)	1,41 (1,24 - 1,67)	1,05 (0,94 - 1,17)	1,04 (0,85 - 1,27)
TsMSCGrA	800	3,95 ± 0,31	0,61 (0,56 - 0,67)	1,61 (1,39 - 1,95)	1,05 (0,94 - 1,18)	1,19 (1,96 -1,47)
TsMTPoM	880	3,25 ± 0,31	0,64 (0,57 - 0,71)	2,06 (1,76 - 2,56)	1,10 (0,96 - 1,25)	1,52 (1,21 - 1,91)
TsMSTeC	560	4,27 ± 0,42	0,69 (0,61 - 0,77)	1,69 (1,48 - 2,03)	1,19 (1,04 - 1,35)	1,24 (1,01 - 1,53)
TsGOFiC	1040	3,12 ± 0,20	0,70 (0,63 - 0,78)	2,37 (1,99 - 2,94)	1,20 (1,05 - 1,37)	1,75 (1,38 - 2,21)
TsMTSJPoS	700	5,72 ± 0,52	0,70 (0,66 - 0,75)	1,37 (1,23 - 1,57)	1,20 (1,09 - 1,32)	1,01 (0,84 - 1,20)
TsMSRoE	800	4,23 ± 0,37	0,70 (0,63 - 0,77)	1,73 (1,53 - 2,04)	1,21 (1,07 - 1,36)	1,28 (1,05 - 1,55)
TsGOSLMBeE	700	4,18 ± 0,43	0,71 (0,65 - 0,76)	1,75 (1,51 - 2,18)	1,21 (1,08 - 1,35)	1,29 (1,03 - 1,62)
TsGOGGoS	700	5,36 ± 0,48	0,75 (0,70 - 0,80)	1,55 (1,37 - 1,79)	1,29 (1,17 - 1,42)	1,13 (0,94 - 1,36)
TsBACarfB	800	4,48 ± 0,36	0,77 (0,72 - 0,83)	1,80 (1,60 - 2,11)	1,32 (1,20 - 1,47)	1,33 (1,10 - 1,61)
TsMSATaJ	700	3,15 ± 0,35	0,78 (0,70 - 0,86)	2,60 (2,09 - 3,61)	1,33 (1,17 - 1,51)	1,91 (1,42 - 2,57)
TsBACariM	960	3,60 ± 0,29	0,81 (0,74 - 0,87)	2,31 (2,01 - 2,79)	1,38 (1,24 - 1,53)	1,71 (1,38 - 2,11)
TsMGLoR	800	3,47 ± 0,31	0,84 (0,77 - 0,92)	2,53 (2,14 - 3,17)	1,45 (1,29 - 1,62)	1,86 (1,47 - 2,36)
TsMSDoM	560	4,05 ± 0,37	0,85 (0,78 - 0,92)	2,17 (1,87 - 2,66)	1,45 (1,30 - 1,62)	1,60 (1,28 - 1,99)
TsGOPoE	880	2,89 ± 0,24	0,86 (0,76 - 0,96)	3,20 (2,70 - 4,02)	1,48 (1,29 - 1,69)	2,36 (1,86 - 2,99)
TsBANReA	800	3,85 ± 0,32	0,86 (0,80 - 0,93)	2,31 (2,00 - 2,81)	1,48 (1,33 - 1,64)	1,71 (1,37 - 2,12)
TsBAPaR	900	3,53 ± 0,27	1,02 (0,90 - 1,15)	3,00 (2,62 - 3,56)	1,75 (1,52 - 2,02)	2,21 (1,80 - 2,72)
TsTOAToM	800	3,15 ± 0,39	1,04 (0,87 - 1,18)	3,47 (2,94 - 4,48)	1,79 (1,51 - 2,11)	2,56 (2,01 -3,26)
TsBARCoB	700	5,75 ± 0,49	1,16 (1,10 - 1,23)	2,25 (2,03 - 2,60)	1,99 (1,81 - 2,18)	1,66 (1,38 - 1,99)
TsBAMaS	900	3,28 ± 0,24	1,46 (1,33 - 1,58)	4,62 (4,00 - 5,56)	2,49 (2,23 - 2,78)	3,41 (2,76 - 4,21)
TsBAMuB	560	3,55 ± 0,29	1,59 (1,44 - 1,74)	4,62 (3,92 - 5,73)	2,71 (2,40 - 3,06)	3,40 (2,69 - 4,30)

Low levels of resistance to deltamethrin estimated for *T. brasiliensis* are promising and could be explained by the massive introduction of susceptible genes of triatomines from wild environment. After the introduction of new susceptible individuals in the domiciliary populations, especially from wild triatomines, which never had contact with pesticides, genetic exchange could cause a decrease in the frequency of resistant phenotypes. One must consider the persistence of populations of *T. brasiliensis* in the natural environment, which disperse quickly and easily to the artificial environment. It is suggested that the spatial mobility can powerfully influence the introduction of susceptible genes contributing in the reduction of resistant genotypes. We conclude that levels of resistance to deltamethrin found for *T. brasiliensis* and *T. sordida* were low (RR_{50} <3) in 31 municipalities of 10 states evaluated, following a pattern similar to those of reference populations in terms of susceptibility. The use of pyrethroids may continue in the study area, yet it is necessary to monitor temporal changes in susceptibility to properly guide the actions of vector control of Chagas Disease.

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