ACTA
AMAZONICA

Analysis of toxicity on *Bacillus sphaericus* from amazonian soils to *Anopheles darlingi* and *Culex quinquefasciatus* larvae

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ABSTRACT

Bioassays under laboratory conditions aiming to determine the larvicidal activity of *Bacillus sphaericus* were carried out on *Anopheles darlingi* and *Culex quinquefasciatus*. In order to estimate the toxicity through median lethal concentration (LC_{50}) and the relative potency of the strains to *B. sphaericus* standard strain 2362, probit analysis was performed utilizing the POLO-PC program. The findings of LC_{50} pointed out high effectiveness on strains IB15 (0.040 ppm), IB19 and S1116 (0.048 ppm), IB16 (0.052 ppm) and S265 (0.057 ppm). Strain IB15 presented nearly 50% more potency than strain 2362 in bioassays conducted on *A. darlingi*. It was observed that IB16 and S1116 strains were the most powerful against *C. quinquefasciatus*, showing to be about 300-400% stronger than 2362 strain. The results show that laboratory conditioned evaluation can be an important way to select promising bacteria with entomopathogenic action on biolarvicides production for use on mosquitoes breeding sites.

KEYWORDS: Vector control, Bioassays, Biolarvicides, Amazonian, Entomopathogens.

Análise da toxicidade em *Bacillus sphaericus* de solos da Amazônia em larvas de *Anopheles darlingi* e *Culex quinquefasciatus*

RESUMO

Bioensaios sob condições de laboratório foram realizados em larvas de *Anopheles darlingi* e *Culex quinquefasciatus*, visando determinar a atividade larvicida de *Bacillus sphaericus*. Para estimar a toxicidade através da concentração letal mediana (CL_{50}) e a potência das estirpes em relação à estirpe padrão 2362, foi realizada a análise de probit utilizando o programa POLO-PC. Os resultados da CL_{50} apontaram alta efetividade para as estirpes IB15 (0,040 ppm), IB19 e S1116 (0,048 ppm), IB16 (0,052 ppm) e S265 (0,057 ppm). A estirpe IB15 apresentou potência cerca de 50% maior que a estirpe 2362 nos bioensaios realizados com *A. darlingi*. Foi observado que as estirpes IB16 e S1116 foram as mais tóxicas para controle de *C. quinquefasciatus*, mostrando-se cerca de 300-400% mais potente. Os resultados mostram que a avaliação em laboratório é uma importante etapa para selecionar bactérias com ação entomopatogênica a serem usadas na para a produção de biolarvicidas para uso nos criadouros das larvas de mosquitos.

PALAVRAS-CHAVE: Controle de vetores, Bioesnasios, Biolarvicidas, Amazônia, Entomopatógenos.

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INTRODUCTION

The intensive use of chemical products for controlling insects down through the years has confirmed their negative impact on the environment. This can be observed by the seriously damaged natural conditions as well as by the increased resistance to these products by insect populations (Tadei, 2001). In compensation, the use of entomopathogenic bacteria has been consolidating itself as a feasible alternative for the integrated control of vectors (Becker, 2003; Tadei & Rodrigues, 2002).

A program on biological control of mosquitoes, virulence prospecting and evaluation of new isolates around the world is one of the most important steps taken to determine their effect on target populations, and thereby selecting the most promising ones for producing biological insecticides.

In Brazil, investigating activities carried out with *Bacillus sphaericus* in several regions of the country has made the discovery of high toxicity bearing strains possible (Schenkel *et al.*, 1992; Vilarinhos *et al.*, 1996; Rodrigues *et al.*, 1999; Litaiff, 2002; Silva *et al.*, 2002). Nevertheless, there is little information regarding the effects of Amazonian strains on vector-borne diseases, such as malaria which represents a severe problem in the region, with a yearly average of about 500,000 cases, accounting for 99.7% of those registered in Brazil (FUNASA/DIVEP/SISMAL). Hence, this study aims to evaluate the toxicity of *B. sphaericus* in several Amazonian locations on *Anopheles darlingi* and *Culex quinquefasciatus* larvae, to establish dose–response lines against susceptible vector species, to select the most powerful ones as biological control agents, and to contribute towards implementing a strategy for controlling vectors in the region.

MATERIAL AND METHODS

The isolation of *B. sphaericus* was performed according to the World Health Organization (2005) from soil samples collected in different localities in Amazonia (Table 1). Soil samples were mixed in NaCl (0.85%) solution and submitted to thermal shock (80°C, 12 min; ice, 5 min). Aliquots of the solution were placed on plates in a nutrient agar medium (meat extract 3 $g.l^{-1}$, peptone 5 $g.l^{-1}$, and agar 15 $g.l^{-1}$) and incubated at 30°C for 48 h. Colonies were identified by morphology of spores and by observation on a phase contrast light microscope. Later, the cultures of 10^8 spores. ml^{-1}, obtained on standardized growth on NYSM medium (Myers & Yousten, 1978), were lyophilised before use in the bioassays. Strains from the remaining states in Northern Brazil were provided by the CENARGEN/EMBRAPA culture collection, including the 2362 strain (Weiser, 1984) which was used as standard.

Table 1 - *Bacillus sphaericus* strains isolated from soil samples of diverse localities of Amazonia.

Strain	Provenance	Origin
IB05	Tarumãzinho - Manaus, AM	1
IB07	Janauarilândia - Manaus, AM	1
IB08	Coari, AM	1
IB09	Coari, AM	1
IB10	Tupé - Manaus, AM	1
IB11	Presidente Figueiredo, AM	1
IB12	Brasileirinho - Manaus, AM	1
IB15	Janauarilândia - Manaus, AM	1
IB16	Tarumã - Manaus, AM	1
IB17	Puraquequara - Manaus, AM	1
IB18	Presidente Figueiredo, AM	1
IB19	Tarumanzinho - Manaus, AM	1
S265	Belém, PA	2
S323	Boa Vista, RR	2
S579	Boa Vista, RR	2
S589	Boa Vista, RR	2
S594	Boa Vista, RR	2
S662	Lagoa Cacoal Grande, PA	2
S841	Belém, PA	2
S1116	Itaubal, AP	2
2362*	Nigéria	3

1 Isolated on Malaria and Dengue Laboratory.
2 Provided by the CENARGEN/EMBRAPA.
3 Provided by Institute Pasteur.
*Standard strain

Twenty strains of *B. sphaericus* was tested on the third instar *A. darlingi* and *C. quinquefasciatus* larvae cultivated at 26 ± 2°C, relative humidity above 85% and photoperiod of 12L:12D according to Scarpassa & Tadei (1990).

Bioassays were carried out by testing seven doses: 1.00 ppm, 0.50 ppm 0.25 ppm, 0.12 ppm, 0.06 ppm 0,02 ppm and 0.01 ppm, obtained from successive dilutions of stock solution of lyophilised *Bacillus* culture according WHO guidelines (WHO, 2005). In each dose, five replicates of plastic cups were set up containing distilled water, 20 late third instar larvae and bacteria doses. In each cup was added 1 ml of food (1 g of fish flour and 8 g of liver flour diluted on 1000 ml of destiled water). The final volume in each cup was 100 ml.

Control groups were set up under the same conditions, but without spores application. Bioassays were performed in three replications on different days, totalling 2,100 larvae per strain. Monitoring was conducted at 24 and 48 h intervals following the *Bacillus* application, when readings of live and dead larvae were made. The bioassays were held at 26 ± 2°C, and a photoperiod of 12L:12D (Dulmage *et al.*, 1990). Bioassays, where the control group showed mortality between 5-10%, were corrected by the Abbott formula (Finney, 1971)

Mortality data was analysed if variance (ANOVA) and average mortality rates in the three bioassays, including the control group, were compared by Tukey's test at 0.05 probability level. Mean lethal concentrations (LC50) at 95% confidence intervals and relative potency to standard strain 2362 were obtained through the Probit analysis (Finney, 1971), utilising the POLO-PC program (LeOra Software), which tests the linearity of dose responses and estimates slopes.

RESULTS

Twenty *B. sphaericus* strains were studied: twelve strains isolated in soil samples from the state of Amazonas, eight strains were provided by the CENARGEN/EMBRAPA from the entomopathogenic collection. As standard strain, was used the *B. sphaericus* 2362.

Overall, 100,800 larvae were used in bioassays with *A. darlingi* and *C. quinquefasciatus*, with nearly 90% of the larvae mortality occurring within 24 h. The highest susceptibility was found in *C. quinquefasciatus*, with larvae mortality variance at 24 h ($F = 316.47$; $P < 0.001$) and 48 h ($F = 299.58$; $P < 0.001$). Strains IB15, S1116, IB19 (average of 65.8 %) presented the highest mortality percentiles in *A. darlingi* larvae and strains IB16, S265, S1116, IB10, IB15, IB12, S594, IB19, S580, and IB08 (74.4 %) in *C. quinquefasciatus,* considering the 24 h reading. In 48 h, IB15, IB19, and S1116 (69.0 %) strains were the most powerful in the bioassays with *A. darlingi* and IB16, S1116, S265, and IB10 (82.8 %) in the bioassays with *C. quinquefasciatus* (Table 2).

These findings are reflected in the LC_{50} values with confidence interval at 95% (Table 3). The 24 h reading was considered for the analysis on account of its high mortality index (>90%). With *A. darlingi* the greatest effectiveness was found in IB15 with 0.040 ppm (0.034-0.047), which was statically significant; S1116 and IB19, 0.048 ppm (0.039-0.069); in IB16, 0.052 ppm (0.045-0.060); and S265, 0.057 ppm (0.051-0.064), however, with no statistically significant difference for the findings with 2362, 0.057 ppm (0.047-0.069). Lower effect was found in IB18, 0.864 ppm (0.625-1.312); IB12, 0.617 ppm (0.527-0.737); and S579, 0.524ppm (0.453-0.616). In the bioassays with *C. quinquefasciatus,* the most effective strains were IB16, 0.014 ppm (0.012-0.016); S1116, 0.016 ppm (0.014-0.018); S265, 0.017 ppm (0.014-0.019) IB10 and IB19, 0.018 ppm (0.014-0.022); IB12, 0.024 ppm (0.021-0.072); and IB15, 0.025 ppm (0.020-0.030). We were able to reach LC50 of 0.065 ppm (0.059-0.072) with the standard strain 2362. this comparison among larvae mortality relative to 2362 strain to *A. darlingi* and LC_{50} values is summarized on Figure 1.

As for standard strain 2362, four of the examined isolates presented greater relative potency in bioassays with *A. darlingi*: IB15 (1.515), S1116 (1.244) and IB19 (1.238), IB16 (1.156). With *C. quinquefasciatus*, higher potency was ascertained in 17 isolates, while IB16, S1116, S265, and IB19 were about three to four times superior (Table 4).

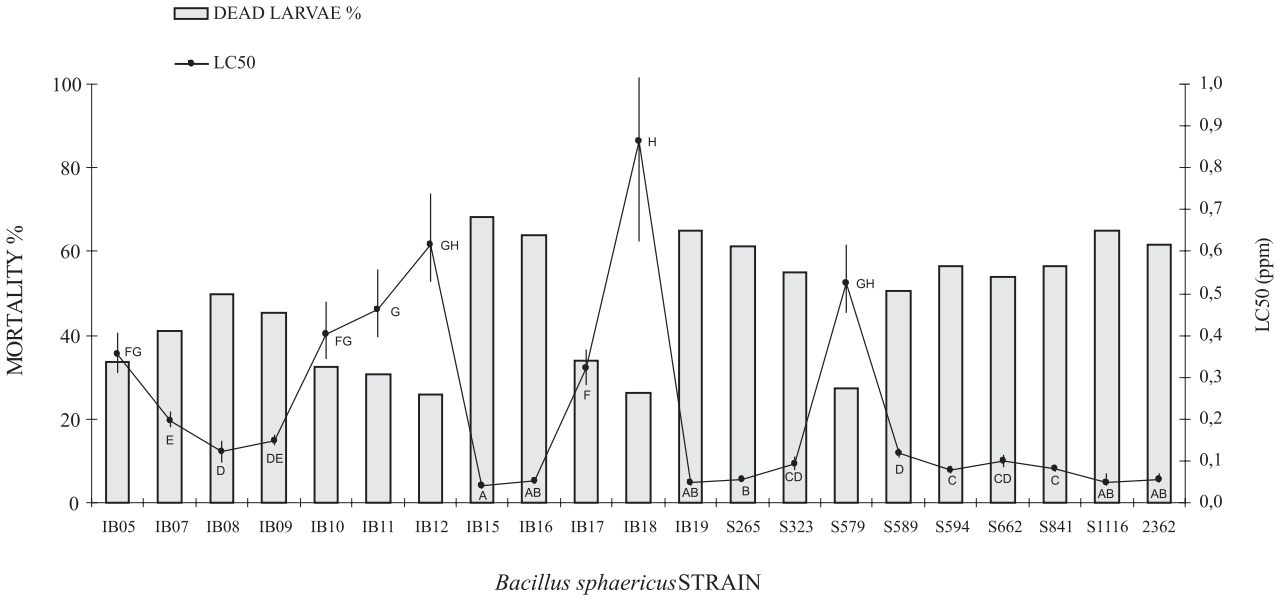

Figure 1 - Median lethal concentration and mortality of third instar larvae of *Anopheles darlingi* obtained from bioassays carried out with *Bacillus sphaericus* strains. (Equal letters do not differ statistically between each other $P < 0.05$).

Table 2 - Mean mortality (± SD) of late third instar larvae obtained from bioassays with *Bacillus sphaericus*.

Strain	24 h		48 h	
	Anopheles darlingi	*Culex quinquefasciatus*	*Anopheles darlingi*	*Culex quinquefasciatus*
IB15	68.09 ± 1.98 aCD	74.95 ± 3.41 bB	70.71 ± 1.51 aBC	77.90 ± 0.22 bB
S1116	65.04 ± 0.46 aCD	81.19 ± 8.67 aAB	68.01 ± 1.75 abC	83.72 ± 1.45 abA
IB19	64.95 ± 0.22 aCD	78.38 ± 1.54 abAB	68.19 ± 1.25 abC	81.85 ± 1.03 bAB
IB16	64.00 ± 0.50 abCD	82.38 ± 1.90 aA	66.33 ± 1.51 abCD	84.67 ± 1.80 aA
S265	61.19 ± 0,46 bD	80.14 ± 1.00 aAB	64.38 ± 1.68 bCD	82.81 ± 2.80 abA
S841	56.57 ± 0,14 cDE	61.43 ± 0.50 dD	60.95 ± 1.08 bcCD	64.81 ± 0.87 dCD
S594	56.37 ± 1.30 cDE	66.00 ± 0.55 cdCD	59.67 ± 2.41 bcD	68.29 ± 0.14 cdC
S323	55.09 ± 1.05 cDE	72.19 ± 2.49 bcBC	58.71 ± 5.15 bcD	74.29 ± 0.85 bcBC
S662	53.81 ± 1.11 cE	59.72 ± 1.41 dD	56.90 ± 1.66 cD	64.38 ± 1.46 dCD
S589	50.47 ± 1.41 dE	69.81 ± 1.39 bcBC	53.62 ± 1.43 cdDE	74.09 ± 3.43 bcBC
IB08	49.80 ± 2.11 dE	68.24 ± 0.58 cC	54.00 ± 0.94 cdDE	73.76 ± 1.36 cBC
IB09	45.52 ± 0.22 eE	63.76 ± 1.20 cdCD	48.76 ± 0.91 dE	67.24 ± 0.79 cdC
IB07	41.04 ± 1.15 fF	62.14 ± 1.68 dD	45.10 ± 1.30 deE	68.57 ± 1.21 cdC
IB17	34.04 ± 1.05 gF	22.38 ± 0.46 gH	39.47 ± 1.66 eEF	26.43 ± 1.75 fGH
IB05	33.28 ± 0.57 gF	61.00 ± 1.31 dD	39.10 ± 1.56 eEF	67.33 ± 2.08 cdC
IB10	32.62 ± 1.30 ghFG	75.86 ± 0.93 abB	36.05 ± 1.36 eF	80.00 ± 0.51 abAB
IB11	30.60 ± 0.54 hG	54.14 ± 1.40 eE	33.72 ± 0.62 efF	59.90 ± 2.70 dD
S579	27.28 ± 1.25 iGH	18.33 ± 1.72 gI	33.86 ± 2.62 efF	22.57 ± 1.08 fH
IB18	26.33 ± 0.95 iH	31.62 ± 3.25 fG	30.19 ± 1.03 fG	37.33 ± 1.05 eEF
IB12	25.90 ± 0.86 iH	73,81 ± 0,42 bB	28.76 ± 1.92 fGH	77.90 ± 0.73 bB
2362	61.66 ± 0.30 bD	59.72 ± 2.35 dD	65.09 ± 1.46 abCD	63.66 ± 0.93 dCD
Control	1.19 ± 0.43 gI	1.70 ± 0.40 gI	1.26 ± 0.58 gI	1.75 ± 0.58 gI

Each bioassay: 2,100 larvae tested per strain.
Means followed by the same letter are not significantly different (Tukey, $P < 0.05$). Capital letters, comparisons between the columns. Small letters, comparisons between lines.

Isolates IB15, IB19 and S1116 were compared for their pathogenicity to 2362 strain, and the findings graphically represented in Figure 2(A-F). Stains IB15, IB16, S1116 show themselves to be more efficient than standard strains, and even more effective than IB19 and S1116. These last two presented the same toxicity as confirmed in the coinciding straight lines.

DISCUSSION

The high mortality rate of larvae within a time interval lower than 24 h observed on the tests with entomopathogenic bacteria, which is mainly observed in dipterous aquatic larvae, confirms one advantage from use of this bacteria in fast response time, namely when compared with terrestrial injurious insects.

Table 3 - Median lethal concentration (LC_{50} ppm) of *Bacillus sphaericus* strains to third instar larvae of *Anopheles darlingi* and *Culex quinquefasciatus*.

Strain	*Anopheles darlingi*		*Culex quinquefasciatus*	
	Probit equation	LC_{50} ppm (95% CI)	Probit equation	LC_{50} ppm (95% CI)
IB15	Y = 2.466 + 1.771x	0.040 (0.034 – 0.047)	y = 2.586 + 1.616x	0.025 (0.020 – 0.030)
S1116	Y = 2.148 + 1.627x	0.048 (0.040 – 0.069)	y = 3.011 + 1.680x	0.016 (0.014 – 0.018)
IB19	Y = 2.157 + 1.637x	0.048 (0.039 – 0.057)	y = 2.512 + 1.441x	0.018 (0.014 – 0.022)
IB16	Y = 2.176 + 1.695x	0.052 (0.045 – 0.060)	y = 2.951 + 1.588x	0.014 (0.012 – 0.016)
S265	Y = 1.742 + 1.104x	0.057 (0.051 – 0.064)	y = 2.792 + 1.576x	0.017 (0.014 – 0.019)
S841	Y = 2.157 + 1.984x	0.082 (0.074 – 0.089)	y = 1.911 + 1.557x	0.059 (0.053 – 0.066)
S594	Y = 1.695 + 1.536x	0.079 (0.071 – 0.087)	y = 2.706 + 2.060x	0.049 (0.044 – 0.053)
S323	Y = 2.473 + 2.410x	0.094 (0.079 – 0.109)	y = 2.382 + 1.561x	0.030 (0.026 – 0.033)
S662	Y = 2.028 + 2.033x	0.101 (0.085 – 0.116)	y = 2.456 + 1.583x	0.028 (0.025 – 0.031)
S589	Y = 1.808 + 1.948x	0.118 (0.106 – 0.130)	y = 1.962 + 1.309x	0.032 (0.027 – 0.036)
IB08	Y = 1.339 + 1.454x	0.120 (0.096 – 0.147)	y = 2.213 + 1.559x	0.038 (0.034 – 0,042)
IB09	Y = 1.342 + 1.625x	0.149 (0.135 – 0.164)	y = 2.351 + 1.835x	0.052 (0.046 – 0.059)
IB07	Y = 1.201 + 1.704x	0.197 (0.179 – 0.218)	y = 1.454 + 1.130x	0.052 (0.044 – 0.059)
IB17	Y = 0.632 + 1.278x	0.320 (0.282 – 0.365)	y = 0.184 + 1.046x	0.960 (0.670 – 1.605)
IB05	Y = 0.612 + 1.360x	0.354 (0.311 – 0.405)	y = 1.241 + 1.114x	0.053 (0.046 – 0.060)
IB10	Y = 0.397 + 1,000x	0.401 (0.342 – 0.478)	y = 1.959 + 1.117x	0.018 (0.014 – 0.021)
IB11	Y = 0.345 + 1.031x	0.463 (0.393 – 0.556)	y = 1.087 + 1.012x	0.084 (0.073 – 0.097)
S579	Y = 0.343 + 1.223x	0.524 (0.453 – 0.616)	y = 0.249 + 0.895x	1.899 (1.387 – 2.850)
IB18	Y = 0.543 + 0.858x	0.864 (0.625 – 1.312)	y = 0.120 + 0.568x	0.952 (0.584 – 1.930)
IB12	Y = 0.253 + 1.208x	0.617 (0.527 – 0.737)	y = 2.195 + 1.360x	0.024 (0.021 – 0.028)
2362	Y = 1.481 + 1.484x	0.057 (0.047 – 0.069)	y = 1.855 + 1.564x	0.065 (0.059 – 0.072)

x = log of dose tested.

The *Bacillus* that present entomopathogenic activity brings about a collapse in the nervous and muscle systems, resulting in the loss of ability to fluctuate, and, consequently, asphyxia by drowning becomes the main cause of death (Habib, 1983). Oliveira & Tadei (2005) described the body paralysis as the initial disturbance of larval behaviour of *A. albitarsis, C. quinquefasciatus* and *A. aegypti,* after being treated for 30 minutes with 0.01, 0.1 and 1.0 mg/l of *B. sphaericus* of 2362 and S1116 strains. Changes on internal and external morphology were observed after 15 minutes with evident structural disorganisation of the intestinal epithelium, showing most of the cells to be swollen, vacuolated, with an increased number of secretion vesicles and an irregularly disposed brush border (Oliveira *et al.*, 2005).

In this study, all tested strains presented toxicity, but in differentiated levels in both target-species. Only the IB15 strain showed high toxicity in tests with *A. darlingi,* (larvae mortality above 70%), twelve isolates showed mean toxicity (30 and 70%) and seven showed low toxicity (under 30%). In *C. quinquefasciatus*, ten, seven, and three isolates presented

Table 4 - Potency of *Bacillus sphaericus* strains from Amazonian soils relative to 2362 standard strain obtained from POLO-PC.

	Relative potency to 2362 strain		
Strain	*Anopheles darlingi*	Strain	*Culex. quinquefasciatus*
IB15	1.515 (1.207 – 1.906)	IB16	4.750 (4.081 – 5.553)
S1116	1.244 (1.024 – 1.512)	S1116	4.231 (3.648 – 4.925)
IB19	1.238 (1.008 – 1.523)	S265	3.878 (3.339 – 4.519)
IB16	1.156 (0.942 – 1.420)	IB19	3.435 (2.891 – 4.102)
S265	0.980 (0.829 – 1.160)	IB10	2.910 (2.108 – 4.083)
S841	0.756 (0.570 – 1.000)	IB15	2.640 (2.289 – 3.052)
S594	0.737 (0.640 – 0.849)	IB12	2.484 (2.093 – 2.957)
S323	0.690 (0.465 – 1.020)	S662	2.368 (2.056 – 2.732)
S662	0.620 (0.461 – 0.831)	S323	2.185 (1.895 – 2.524)
S589	0.519 (0.395 – 0.679)	S589	1.891 (1.538 – 2.333)
IB08	0.477 (0.390 – 0.582)	IB08	1.711 (1.488 – 1.970)
IB09	0.391 (0.326 – 0.466)	S594	1.453 (1.143 – 1.849)
IB07	0.296 (0.245 – 0.356)	IB09	1.303 (1.087 – 1.564)
IB17	0.182 (0.147 – 0.223)	IB07	1.110 (0.812 – 1.521)
IB05	0.164 (0.136 – 0.195)	S841	1.100 (0.957 – 1.263)
IB10	0.156 (0.099 – 0.233)	IB05	1.081 (0.767 – 1.526)
IB11	0.137 (0.089 – 0.202)	IB11	0.690 (0.454 – 1.038)
S579	0.116 (0.089 – 0.149)	IB18	0.130 (0.037 – 0.327)
IB12	0.101 (0.077 – 0.129)	IB17	0.088 (0.056 – 0.130)
IB18	0,091 (0.048 – 0.154)	S579	0.060 (0.033 – 0.099)

high, mean, and low toxicity respectively. *C. quinquefasciatus* was more susceptible, reaching 66.8% versus 51.5% in *A. darlingi*. These findings agree with those from earlier studies pointing out the high susceptibility of *Culex* sp. to *B. sphaericus* (Singer, 1980; Yousten, 1984; Mulla *et al.*, 1986).

Gujar (2001) had considered that the difference among observed effects in distinct larvae instars treated with *B. thuringiensis* may be explained by less food consumed by later instar larvae, resulting in less absorption efficiency in digesting food, but compensated by an increase in the utilisation of ingested and digested food into body substance. According to Nielsen-LeRoux (1992) and Silva-Filha (2005), the differentiated activity of the toxin in insects may be also attributed to the affinity of the receptors present in the intestinal epithelium with toxin among some mosquito species. The description of membrane receptors and their interaction with the toxin contribute to elucidate how the *B. sphaericus* and resistance mechanisms act.

Pathogenicity tests among new isolates are essential in order to select strains for the production of biolarvicides as well as to estimate the virulence in commercial products. Laboratory bioassays determine the *Bacillus* minimum effective dose, as a parameter for use in the field (Becker, 2003). In the assays carried out with *C. quinquefasciatus,* eleven strains presenting LC_{50} between 0.014 and 0.038 ppm were significantly more efficient than the results obtained from strain 2362 (0.065 ppm). On *A. darlingi* only IB15 (0.040 ppm) was more effective than the standard strain (0,057 ppm). Similar findings were obtained by Lacey & Singer (1982) in their tests with *B. sphaericus* 2013-4 and 2013-6. These authors obtained LC_{50} equal to 0.0187 and 0.0168 ppm on *A. albimanus* and LC_{50} of 0.0527 and 0.0558 ppm on *A. quadrimaculatus* larvae.

In relation to the standard strain, IB15 was nearly 50% more efficient, followed by S1116 and IB19 (24%). On *C. quinquefasciatus,* isolates IB16, S1116, S265, and IB19 were 300-400% more powerful. Other works with *B. sphaericus* indicated strains with greater potency than the 2362 in bioassays with *A. nuneztovari* and *A. darlingi,* S20, S46, S2, and S4, with a potency four and five times greater (Rodrigues *et al.*, 1998).

In Brazil, several isolates from all regions of the country have shown great potential for use in biolarvicides, but due to differences in methodology employed in bioassays, these findings cannot be compared with those found in the present study (Silva *et al.*, 2002; Monnerat *et al.*, 2004).

Comparison between IB15, IB19, and S1116 Probit lines, showed parallel lines, indicating qualitative similarity; however, it was necessary to use a smaller dose of the *Bacillus* to kill 50% of the target population in the three Amazonian strains. In one to one comparisons, IB15 presented a greater effectiveness than IB19 and S1116; the two were similar as indicated by the coinciding straight-lines.

Larvicidal activity was observed in all strains of *B. sphaericus* from Amazonia in differentiated toxicity levels, while the *C. quinquefasciatus* larvae was more susceptible than *A. darlingi*. Strains IB15, IB19, and S1116 showed greater relative potency to the standard strain 2362, and are recommended as potential agents for the biological control of mosquitoes. In field trials, the diversity in larvicidal activity plus the ecological effects are relevant when considering the possibilities of using *B. sphaericus* for the biological control of mosquito target-species that coexist in breeding sites, and the laboratory bioassays are a good tool for screening entomopathogenic microrganisms.

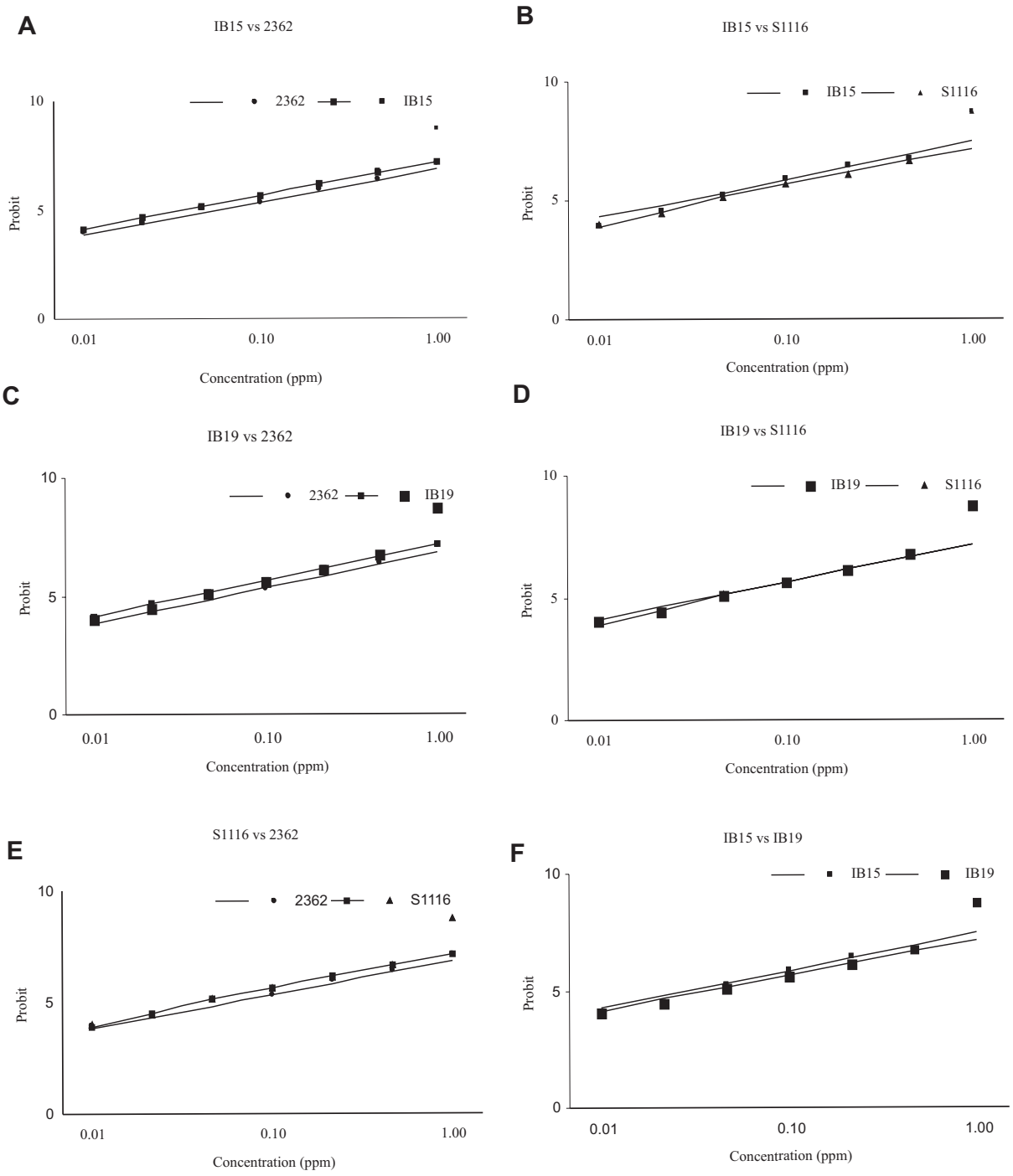

Figure 2 - Comparison among *Bacillus sphaericus* stains linear regression lines in tests with *A. darlingi* larvae.

ACKNOWLEDGEMENTS

The authors thank FAPEAM, CTPETRO and PIATAM for financial support on this study; Dr Rose Gomes Monnerat (CENARGEN/EMBRAPA) for providing some *B. sphaericus* strains; and the Malaria Dengue Laboratory' technicians for collecting and identifying the mosquitoes species.

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Recebido em 10/05/2007
Aceito em 19/03/2008