Mosquito Control Based on Larvicides in the State of Rio Grande do Sul, Brazil: Choice of the Control Agent[1]

Controle de Mosquitos com Base em Larvicidas no estado do Rio Grande do Sul, Brasil: A Escolhas do Agente de Controle

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A comparison between chemical and biological larvicides in routine operations against mosquitoes in Rio Grande do Sul State was carried out in this study.

In laboratory bioassays against Culex quinquefasciatus, biological formulations Vectobac 12 AS and Teknar 3000 (Bacillus thuringiensis israelensis) as well as ABG 6262 (B. sphaericus 2362), both in liquid and powder form, were highly effective. Locally produced B.thuringiensis israelensis, formulations also yielded good results. Among chemical larvicides, pyrethroid compounds Pirisa and K-Othrine yielded better results than the organophosphates Lebaycid and Abate. These last formulations yielded responses ten weaker than predicted in other studies.

Under field conditions, a dose of 1250 mg/m² for biological formulations was considered adequate for routine application because at this level it is possible to overcome physical influences on results. Only B.sphaericus preparations caused important disruption of mosquito colonization in active breeding sites. Up to 39 weeks were tabulated without complete colonization in natural conditions and one month in artificially colonized tanks.

This study suggests that biological alternatives should be considered in mosquito control programs. They may be a solution to such problems as resistance to larvicides, elimination of natural enemies, and short-lasting effects of applications.

Key words: *Mosquitoes; Larvicides; Biological Control*

INTRODUCTION

Mosquitoes are major disease vectors as well as nuisance insects and are therefore relevant to control programs in Brazil. Most of these programs still rely on techniques that eliminate adult mosquitoes.

Nevertheless, recent concerns related resistance, environmental damage, and safety for operators have stimulated a discussion on alternate methods for controlling these vectors and have highlighted the importance of actions against mosquito larvae and their breeding sites (Ruas-Neto & Oliveira, 1985).

Methods for using larvicides have not been extensively studies in recent years in Brazil and important problems such as choice of the agent and application procedures have not been resolved. The present study, carried out in the State. Rio Grande do Sul, analyzes some of the advantages and disadvantages of several larvicides agents (Ruas-Neto & Silveira, 1989).

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LABORATORY TESTS AGAINST MOSQUITOES

Laboratory bioassays were carried out from July 1989 to March 1991. They employed laboratory reared speciment of *Culex quinquefasciatusa* Say, the target species for nearly all local control programs in Rio Grande do Sul (Ruas-Neto, 1989).

Rearing techniques used for *C. quinquefasciatus* were similar to those described in a previous study, with some adaptations (Ruas-Neto & Silveira, 1989). The optimal diet for larvae was a mixture of ground concentrated catfood pellets, yeast powder, and rabbit dung at a ratio of 7:2:1. A fourteen-hour period of light was supplied, but temperature was not completely stable. Adults were fed on common sugar and weekly blood meals were obtained from quails (*Nothura maculosa*).

Early four-instar larvad (5 mm) were always used for tests. Selected individual results were pooled and then analyzed on a log-probit basis. The number of replicates for each formulation was not determined. Individual results with close standard lethal concentrations (LC50 and LC90) were selected. For each group of tests, the variation coefficient of lethal concentration values was less than 20%.

Formulations used in the tests are shown in Table 1. Dilutions were always prepared immediately before use and readings were done after 24 hours.

TABLE 1. Larvicides Formulations

Name	Code	Presentation	Main Component	Potency
VECTOBAC 12AS	V	Suspension	*B.t.i.*⋆	1200
SKEETAL	S	Suspension	*B.t.i.*	*600*
ABG 6262	A6262R (room temp.A)	Suspension	*B.s.* 2362⋆⋆	–
ABG 6262	A6262L (low temp.)	Suspension	*B.s.* 2362	–
ABG 6262	A6262P	Powder	*B.s.* 2362	–
ABG 6234	A6234	Suspension	*B.s.* 2362	–
DEB	D	Wettable powder	*B.t.i*	1500
PIRISA	P	Solution	Tetramethrine/PBO	1%/9%
LEBAYCID	L	Solution	Fenthion	50%
K-OTHRINE	K	Solution	Deltamethrine	2.5%
ABATE	A	Solution	Temephos	50%
BTI-SSMA	B-SSMA	Suspension	*B.t.i.*	–
FIOCRUZ 5A	F-5A	Cream	*B.t.i.*	–
FIOCRUZ 29C	F-29C	Cream	*B.t.i.*	–
FIOCRUZ BACTIS	F-BC	Suspension	*B.t.i.*	–
TEKNAR	T	Suspension	*B.t.i.*	3.000

⋆ *Bacillus thuringiensis israelensis*
⋆⋆ Bacillus sphaericus

Note: Potency in ITU/mg for biological formulations or % active ingredients for chemical formulations.

FIELD TESTS

Field tests were carried out in locations described in Table 2, from August 1989 and December 1990. For determination of immediate responses, tests were carried out either using netted cylinders (1 m high,0.5 m wide with 1 mm mesh) to limit the area, or over the entire experimental area. The density unit used was the mean number of larvae per dip of 200 ml – MLPD. Dips were always taken along the margins to ensure uniformity of

sample. Reductions in this mean were used as estimates of larval mortality.

Larvae were sampled and sifted through a nylon sieve (1 mm mesh) to retain L3 and L4 larvae, which were then used for calculation of MLPD. Dips were taken until the variation coefficient of larvae per dip counts was less than or equal to 20%. MLPD values were compared as anti-log means of log-transformed counts in every test (Elliot, 1983).

Tests over entire experimental areas were evaluated according to observed reduction of MLPD in the area or %RT, after 24 hours as follows:

%RT: {(SR/IR) x 100} – 100 where,
IR: initial reading of MLPD in entire area (before treatment).
SR: sequential reading of MLPD in entire area (after treatment).

TABLE 2. Sites for Field Tests

Code	Description	Species Composition	Districts
1	Sewage ditch (100 x 82 x 0.5 m)	*C. quinquefasciatus*	Porto Alegre
2	First daity industry lagoon (200 x 50 x 0,4 m)	Suspension	Teutonia
2*	Second dairy industry lagoon (200 x 50 x 0.4 m)	*C. saltanensis*	Teutonia
3	Permanent pond at a pigpen (30 x 50 x 0.5 m)	*C. inflictus, C. saltanensis, C. coronator, C. quinquefasciatus*	Feliz
4	Rain pond in a garbage deposit (5 x 12 x 0.2 m)	*C. coronator, C. saltanensis C. bigoti*	Feliz
5	First rain pond in a yard (1 x 0.5 x 0.2 m)	*C. quinquefasciatus, C. bigoti*	Feliz
6	Second rain pond in a yard (10 x 30 x 0.25 m)	*C. inflictus, C. quinquefasciatus*	Feliz
7	Rain pond at a pigpen (2.5 x 1.5 x 1 m)	*C. quiquefasciatus, C. bigoti*	Feliz
8	Slow part of a shallow stream (0.8 x 1 x 0.2 m)	*Anopheles argyritarsis, Aedomyia* sp., *Culex* sp.	Tenente Portela
9	Rain pond in a field (20 x 10 x 0.1 m)	*Anopheles albitarsis, Aedomyia* sp., *Culex* sp.	Tenente Portela
10	First rain pond in a forest (4 x 2 x 0.2 m)	*Psorophora* sp. *Aedes crinifer*	Tenente Portela
11	Second rain pond in a forest (4 x 2 x 0.2 m)	*C. coronator, Aedes crinifer, Psorophora* sp.	Tenente Portela
12	Tank artificially infested (250 l)	*C. inflictus. C. saltanensis, C. coronator, C. quiquefasciatus*	Feliz

* *Culex*

Most field applications were carried out using a hand-operated rack sprayer – capacity of 20 l – in which the sprayed volume per area was 34 ml/m^2. In some areas a motor sprayer was used. This had a capacity of 150 l and a sprayed volume per area of 119 ml/m^2.

When control cylinders were used, a correction of reduction of MLPD values was used (Obeta, 1986):

%RT = reduction in MPLD in treated cylinder;
%RT = {(C1/T1 x T2/C2) x 100} – 100. where:
C1 = MLPD in control cylinder before treatment;
T1 = MPLD in treated cylinder before treatment;
C2 and T2 = MLPD after a 24 hour period.

Test with reductions in MLPD in control cylinders over 30% were discarded.

Residual effect of biological larvicides was followed sprayed open areas and in tanks of 250 l, observed in October 1990. Tanks were kept full of field water and received larvae periodically. Twenty dips of 600 ml with larvae were added every day of readings. Immediate reduction in treated tanks was calculated as %RT, similar to cylinder situation. Variation of MLPD in the control tank was observed according to the parameter %RC, or reduction in MLPD considering the first reading. Residual effect in the treated tank was calculated as %RR, a reduction in MLPD in the treated tank considering sequential readings in the control tank:

%RC = {(IRC/SRC) X 100} – 100, where:
IRC = INITIAL MLPD in control tank.
SRC = sequential reading in control tank.
%RR = {(SRT/SRC) x 100} – 100, where:
SRT = sequential reading in treated tank.

RESULT

Table 3 presents probit analysis of bioassays carried out during spring and summer seasons. A temperature effects observed during colder seasons was not analyzed (Ruas-Neto, 1987).

Table 4 presents results observed in field tests with cylinders, were doses are mg of product per arca. Chemical larvicides have doses presented as active ingredient per arca. Estimates of concentrations could be obtained considering a depth range of 0.1 to 0.5 m in each site. Table 5 presents immediate results of field tests with biological larvicides at a dose of 1250 mg/m^2. Residual effect in these sites is shown in Figure 1, as a number of weeks where a reduction of MLPD (%RT) of 90% or higher was observed, compared to initial readings. Table 6 and Figure 2 show results in tanks. Observation of *B. thuringiensis israelensis* application – Vectobac, was discontinued after one week, when the residual effects started decreasing.

TABLE 3. Bioassay Results

Form.	Nº Tests	LC50 +/-s.e. log (anti-log)	LC90 +/- s.e. log (anti-log)	**r**
V	9	1.15 (0.14) +/- 0.008	1.52 (0.33) +/- 0.019	0.9954
S	10	1.42 (0.26) +/- 0.001	1.78 (0.6) +/- 0.18	0.9989
A6262R	8	1.22 (0.17) +/- 0.001	1.73 (0.54) +/- 0.031	0.9750
A6262L	6	1.19 (0.15) +/- 0.001	1.64 (0.44) +/- 0.029	0.9944
A6262P	3	0.76 (0.057) +/- 0.006	1.3 (0.2) +/- 0.042	0.9963
D	6	1.34 (0.22) +/- 0.002	1.82 (0.66) +/- 0.023	0.9973
T	3	1.09 (0.12) +/- 0.004	1.54 (0.35) +/- 0.035	0.9934
B-SSMA	1	1.03 (1.07) +/- 0.018	1.48 (3.02A) +/- 0.87	0.9425
F-5A	1	0.78 (0.06) +/- 0.007	1.13 (0.135) +/- 0.084	0.9819
F-29C	1	0.83 (0.068) +/- 0.016	1.25 (0.178) +/- 0.115	0.9451
F-BC	2	1.38 (0.24) +/- 0.002	1.72 (0.520) +/- 0.04	0.9834
P	4	1.38 (2.4X10^3) +/- 0.003	1.67 (4.7X10^3) +/- 0.034	0.9898
L	3	1.39 (024) +/- 0.003	2.09 (0.12) +/- 0.058	0.9924
K	4	1.21 (1.6X10^3) +/- 0.01	1.79 (6X10^3) +/- 0.049	0.9582
A	5	0.86 (7.4X10^3) +/- 0.001	1 (0.01) +/- 0.11	0.9747

Notes:
Form.: formulations (see codes in table 1)
LC50 and LC90: lethal concentrations plus standard errors calculated after log transformations and anti-log values between brackets.
r: correlation coefficient.

TABLE 4. Field Tests with Cylinders

Form.	Place	Dose	Test	Temp	%RT
P	1	5	1	16	97.87
V	1	1250	1	22	70.41
V	2	1250	2	–	98.48
S	1	1250	1	17	35.07
S	3	1250	2	28	95.44
S	2	1250	3	35	100
S	2	1250	4	34	100
A6234	3	1250	1	28	99.35
A6234	3	1250	2	28	97.76
L	2*	68	1	33	100
D	2*	1250	1	33	100
T	2*	1250	1	22	100
T	2*	312.5	2	29	68.52
T	2*	625	3	29	90.9
T	2*	312.5	4	19	94.66
T	2*	625	5	19	100

Notes:
Test: order of tests for each formulation.
%RT = reduction in MLPD in treated cylinder;
%RT = {(C1/T1 x T2/C2) x 100} – 100
C1 – initial reading of MLPD in control cylinder.
T1 = initial reading of MLPD in treated cylinder (before treatment).
C2 and T2 = reading of MLPD after a 24-hour period
C1, C2, T1, T2 not shown in table.

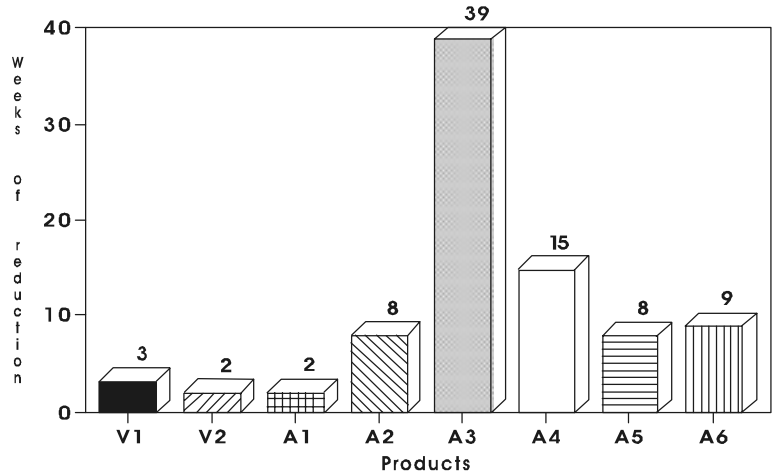
TABLE 5. field Tests in Entire Areas

Form.	Place	Dose	aTest	Temp	%RT
V	4	1250	1	30	100
V	5	1250	2	27	100
V	3	1250	3	17	100
A6234	6	1250	1	31	99.69
A6234	3	1250	2	33	99.37
A6234	6	1250	3	20	100
A6234	5	1250	4	21	100
A6234	4	1250	5	22	100
A6262	7	1250	1	30	99.65
A6262	3	1250	2	28	92.74
A6262	8	1250	3	21	98.23
A6262	9	1250	4	22	100
A6262	3	1250	5	17	99.69
A6262P	10	1250	1	21	94.23
A6262P	11	1250	2	22	96.03
A6262P	12	1250	3	25	100

Notes:

Test: order tests for each formulation.
%RT: reduction in MLPD in entire area.
%RT {(SR/IR) x 100} – 100.
IR: initial reading of MLPD in entire area (before treatment).
SR: sequential reading of MLPD in entire area (after treatment)
IR, SR not shown in table.

FIGURE 1. Residual Effect of *B. thuringiensis israelensis* and *B. sphaericus* in Entire Experimental Areas



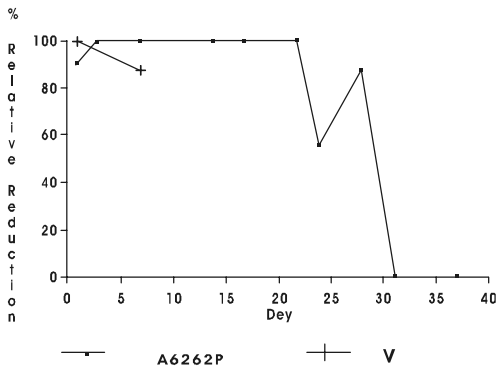
Notes:
Weeks of reduction: period in weeks when %RT was equal or higher than 90%
V1 – Application of Vectobac 12AS in place 4.
V2 – Application of Vectobac 12AS in place 5.
A1 – Application of ABG 6234 in place 6.
A2 – Application of ABG 6234 in place 6.
A3 – Application of ABG 6234 in place 7.
A4 – Application of ABG 6262 in place 3.
A5 – Application of ABG 6262 in place 3.
A6 – Application of ABG 6262 in place 3.

TABLE 6. Residual Effect in Tanks of 250 l

Day	Form	IRC	SRC	IRT	SRT	%RC	%RT	%RR
0-1	A6262P	14.91	12.87	5.65	1.18	13.68	75.80	90.8
3	W/A	–	19.25	–	0.0	–	–	100
7	W/A	–	10.35	–	0.0	–	–	100
14	W/A	–	0.42	–	0.0	–	–	100
17	W/A	–	2.11	–	0.0	–	–	100
22	W/A	–	4.71	–	0.0	–	–	100
24	W/A	–	4.71	–	2.12	–	–	55
28	W/A	–	0.85	–	0.12	–	–	85.9
31	W/A	–	3.43	–	5.12	–	–	S/R
37	W/A	–	3.61	–	18.2	–	–	S/R
0-1	V	45.72	29.33	19.52	0.0	35.85	100	100
7	W/A	–	17.4	–	2.3	–	–	86.8

Notes:
Form.: Formulation.
W/A: Without application.
%RC = reduction of MLPD in control tank.
%RC = {(SRC/IRC) x 100} – 100.
%RT = reduction of MLPD in treated tank.
%RT = {(IRC/IRT x SRT/SRC) x 100} – 100.
%RR = relative reduction of MLPD in treated thank.
%RR = {(SRT/SRC) x 100} – 100.
IRC = initial reading of MLPD in control tank.
IRT = initial reading of MLPD in treated tank (before treatment).
SRC = sequential reading of MLPD in control tank.
SRT = sequential reading of MLPD in treated tank
S/R: no reduction.

FIGURE 2. Residual Effect of *B. thuringiensis israelensis* and *B. spharicus* in Tanks



Notes:
% Relative reduction: see calculation of %RR above.
A6262P – ABG 6262 powder (*B. sphaericus*).
V – Vectobac 12AS (*B. thuringiensis israelensis*).

DISCUSSION

An overall good response was observed in the bioassays with biological formulations Vectobac, Teknar and ABG 6262. Liquid Skeetal and powder DEB produced poorer responses. Experimental *B. thuringiensis israelensis* formulations F-5A and F-29C produced excellent results, suggesting that investment in production of such formulations could be cost-effects (Ruas-Neto & Silveira, 1989).

Pyrethroid larvicides K-Othrine and Pirisa produced results within expectations. In contrast, lethal concentrations of organophosphorates Lebaycid and Abate were considered ten times higher than in other studies, suggesting some tolerance of C. quinquefasciatus to those compounds (Brow, 1986).

Liquid *B. sphaericus* preparations stored at room temperature – A6262R and in the refrigerator – A6262L produced similar results, although heterogeneity of individual tests was much higher in the first case, Further tests are needed to assess the effects of storage temperature.

Table 4 shows that good overall responces to biological formulations under field conditions were obtained at the dose of 1250 mg/m^2. Differences in water quality did not influence the results, except in one situation – a sewage ditch, site I – where some result were poorer. A sharp decrease is expected in the immediate effect at lower doses in organically enriched environments (Sinegre et al., 1980; Mulla et al., 1988; Mulla et al., 1984). Teknar yielded lower reductions in MLPD when doses were 50% or 25% of the above dose. Depth differences did not influence results, suggesting that effectiveness is more related to particle concentrations of the upper layer of the water (Mulla et al., 1988).

Table 6 shows that Vectobac, ABG 6262 and ABG 6262P were lighly effective regardless of the species composition of the site. Predatory *C. (Lutzia) bigoti* was also reduced, probably by eating moribund larvae.

Persistence of biological larvicides in the media has been discussed on several occasions (Lacey et al., 1984; Clark III & Rowley, 1984; Mulla et al., 1984; Balarman et al., 1987). The overall conclusion is that *B. sphaericus* based formulations are superior to *B. thuringensis israelensis* examples. In some *B. sphaericus* strains such as 2362, toxic particles are embedded in the spore and become more protected against physical factors (Karch e Charles, 1987). In *B. thuringiensis israelensis,* toxic particles are crystals released in the medium and therefore less protected. Also, *B. sphaericus* strains produce a higher rate of bacterial multiplication in larval bodies than *B. thuringiensis israelensis,* promoting a partial recycling after released in the medium (Roberts & aGarcia, 1984; Aly et al., 1985).

B. thuringiensis israelensis preparations usually present a short-laasting effect under field conditions (Clark III & Rowley, 1984). Solid slow-releasing formulations may extend this period to two or three weeks (Lacey et al., 1988; Lecy et al., 1984). In contrast, longer periods have been observed with *B. sphaericus* formulations. Periods of one or two months have observed in some studies on *B. sphaericus* persistence (Lacey et al., 1984; Mulla et al., 1984). In this study we observed that *B. sphaericus* effect in artificially

infested tanks persisted for one month, much longer than the *B. thuringiensis israelensis* effect (Table 6 and Figure 2). Under natural conditions, a heavily-infested small body of water, culicines remained controlled for nine months after one *B. sphaericus.* application (the effect in place 7 in Figure 1). The *B. sphaericus* effect probably ends when spores settle to the bottom of the medium (Balarman et al. 1987). Toxic particles of *B. sphaericus* spores are probably destroyed in the mud, but the spores remain viable (Karch & Charles, 1987).

Additional factors that can extend the residual effect of biological larvicides are population interactions and depth of the sites. We observed in some sites that *C. quinquefasciatus* was replaced by less important species after some applications (the effect in site 3 shown in Figure 1). Also, deeper bodies of water appeared to favour persistence of biological larvicides. In contrast, shallow sites or ditches where water is moving probably shorten the effect period.

In some places it is necessary to preserve diversity of organisms, specially those competitors to or predators upon target culicines. In such situations biological larvicides should be chosen. Chemical larvicides ate largely nonselective (Mohsen & Mulla, 1981; Frost & Sinniah, 1982), affecting even vertebrates in some cases (Zinkl et al., 1981). Such larvicides should be used only in places where pollutions is too high to allow for biological diversity, provided that they ate more cost-effective than biological examples.

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RESUMO

RUAS-NETO, A. L.; SILVEIRA, S. M. & COLARES, E.R.C. **Controle de Mosquitos com Base em Larvicidas no Estado do Rio Grande do Sul, Brasil: A Escolha do Agente de Controle.** Cad. Saúde Públ., Rio de Janeiro, 10 (2): 222-230, abr/jun, 1984.

Desenvolveu-se neste estudo uma comparação entre larvicidas químicos e biológicos usados em programas de controle de mosquitos no Rio Grande do Sul.

Em bioensaios de laboratório contra *Culex quinquefasciatus* constatou-se que as formulações biológicas líquidas Vectobac 12 AS e Teknar 3000 (*Bacillus thuringiensis israelensis*), ABG 6262 líquido e em pó (*B. sphaericus* 2362), foram altamente eficazes. Também as formulações experimentais de *B. thuringiensis israelensis* produzidas em laboratórios brasileiros foram consideradas adequadas. Entre as formulações químicas, os compostos piretróides Pirisa e K-Othrine produziram resultados melhores do que os organo-fosforados Lebaycid e Abate. Estes últimos produziram respostas dez vezes mais fracas do que o previsto em outros estudos. Em condições de campo, a dose de 1250 mg/m^2 para as formulações biológicas foi considerada adequada para a rotina das aplicações, porque permite superar as influências físicas do meio sobre os resultados. Somente as formulações de *B. sphaericus* produziram interrupções nas reinfestações dos focos de culicídeos observados. Períodos de até 39 semanas sem reinfestações foram observados em focos naturais e de um mês sem sobrevivência foi observado em tanques, onde procedia-se a reinfestação artificial.

Este estudo sugere que as alternativas biológicas devem ser consideradas em programas de controle de mosquitos. Elas podem superar os problemas de resistência e eliminação, bem como da ausência de efeito residual nas aplicações de larvicidas.

Palavras-Chave: Mosquitos; *Culex* sp; Bacillus *sp;* Larvicidas; Controle Biológico

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