EFFICACY OF AROSURF® MSF AND FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST *ANOPHELES ALBIMANUS*: LABORATORY BIOASSAY[1]

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ABSTRACT. The efficacy of Arosurf® MSF alone and in combination with three preparations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) against *Anopheles albimanus* larvae, pupae and eggs was determined by bioassay. Arosurf MSF alone was effective against the egg, 4th larval instar and pupal stages. All Arosurf MSF and *B.t.i.* combined formulations produced over 90% mortality of all larvae and pupae, 48 hr posttreatment. Egg eclosion was reduced to approximately 25% with all formulations containing Arosurf MSF.

INTRODUCTION

Anopheles albimanus Wiedemann is the primary vector of malaria in Central America and northern South America (Harwood and James 1979), and its control is a major concern in these areas. This species has become physiologically resistant to many of the conventional insecticides (Brown 1986). Along with resistance to many of the synthetic organic insecticides, the potential endangerment to a variety of animal life, including man, by these compounds (Hayes 1975, Matsumura 1975) has created the need for alternative control strategies.

One alternative control strategy is the physicochemical modification of the air-water interface with low doses of non-petroleum monomolecular organic surface films. This type of control strategy has been shown to be effective against certain mosquito species at various life stages, in particular the 4th larval instar and pupal stages (Levy et al. 1980, 1981, 1982a, 1982b; White and Garrett 1977), with no detrimental effects to nontarget organisms (Mulla et al. 1983, Webber 1983). In addition, Levy et al. (1984) have shown that formulations of Arosurf® MSF (Monomolecular Surface Film) with commercial preparations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) (Teknar®, Bactimos® and Vectobac®) and formulations of Arosurf MSF with *B. sphaericus* (Levy et al. 1986) are effective larvicides and pupicides of *Culex quinquefasciatus* Say.

The purpose of this study was to determine the efficacy of Arosurf MSF alone, and in formulation with preparations of *B.t.i.* against *An. albimanus* under laboratory conditions. All formulations were tested for their efficacy as an ovicide, larvicide and pupicide.

MATERIALS AND METHODS

A series of bioassays against the eggs, larvae and pupae of *An. albimanus* were done to determine the efficacy of Arosurf MSF in formulation with three preparations of *B.t.i.* (Teknar, Bactimos and ABG-6193[2]), compared with individual components at their recommended label concentrations. All formulations (Arosurf MSF plus *B.t.i.* combinations and individual components) were bioassayed against each larval instar (1st–4th), pupa and egg stage and replicated three times.

Ten larvae or pupae, or 25 eggs from a laboratory colony of *An. albimanus* were placed into 400 ml sterilized beakers containing 250 ml of deionized water with a dissolved oxygen content of 4.8 mg/liter. All larvae and pupae were newly molted individuals whose ages were less than 12 hr post-molt. Eggs used in the test were 24 hr old. Prior to application of the formulations, larvae were fed three drops of finely ground hogchow-distilled water suspension. All bioassays were done in an environmentally controlled room maintained at 27 ± 1°C and 80 ± 5% RH.

All *B.t.i.* formulations, both singularly and in combination with Arosurf MSF, were prepared as agitated water-based suspensions at an application concentration 0.59 liters/ha (0.5 pint/acre). The potency of the *B.t.i.* was 1,000, 1,200 and 3,000 *Aedes aegypti* International Toxic Units/mg for Bactimos, ABG-6193 and Teknar, respectively. The Arosurf MSF application concentration was 2.43 liters/ha (0.26 gal/acre) for all water-based formulations.

All formulations (Arosurf, *B.t.i.* and combinations) were mixed using a Nuova II® stirrer

[1] The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the view of the Department of the Army or the Department of Defense.

[2] A technical *B.t.i.* formulation developed by Abbott Laboratories, North Chicago, IL 60064.

(Thermolyne Corporation, Dubuque, IA) and magnetic stir bar set at high speed for 1 minute. Formulations were then pipetted into labeled 400 ml beakers containing the various *An. albimanus* life stages at a final concentration of 46.93 liters/ha (5.02 gal/acre), similar to the method described by Levy et al. (1984). The percent mortality of larvae and pupae, along with the percent adult emergence were recorded at 24 hr intervals posttreatment for each formulation. The percentage of egg eclosion was determined as described by Levy et al. (1982a) and recorded.

The cumulative mean percent mortality of each larval instar and pupal stage, in addition to the cumulative percent adult emergence and egg eclosion for each formulation were used as criteria in evaluating their efficacy against *An. albimanus*. The experimental design of this test was a completely randomized design; the data were analyzed by an analysis of variance (ANOVA), and the means were separated using Duncan's multiple range test ($P \leq 0.05$, [SAS/STAT 1985]).

RESULTS AND DISCUSSION

Results of the bioassay indicated that the combined formulations of Arosurf MSF and *B.t.i.* produced a joint larvicidal/pupicidal action on *An. albimanus* that was additive not synergistic when compared with the component formulations (Tables 1–3). No statistical difference was found between any of the combined formulations (Table 2). This joint effect by the combined formulations can be explained by the mode of action of each component (toxic vs. physical) and differences in stage susceptibility (larval vs. pupal) as theorized by Levy et al. (1984).

Egg eclosion was reduced to approximately 25% when eggs were placed into beakers containing either Arosurf MSF alone or in combination with any of the *B.t.i.* components (Table 4). No statistical difference in eclosion was determined between Arosurf MSF alone, or in combination with any of the three *B.t.i.* formulations. Eggs treated with Arosurf MSF immediately sank to the bottom of the beaker, re-

Table 1. Efficacy of water-based formulations of Arosurf® MSF and three preparations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) against 1st–4th instar larvae and pupae of *Anopheles albimanus* 24 hr posttreatment.

Water-based formulation	Cumulative mean percent mortality of each larval instar and pupal stage indicated[a]				
	1st	2nd	3rd	4th	Pupal
Arosurf MSF	16.7 a	12.3 a	20 a	83.3 ab	100 a
Teknar	100 b	100 b	100 b	86.7 ab	6.7 c
Bactimos	100 b	96.7 b	93.3 b	80.0 ab	0 c
ABG-6193	100 b	100 b	100 b	53.3 b	0 c
Arosurf + Teknar	100 b	100 b	100 b	100 a	90 ab
Arosurf + Bactimos	100 b	80 c	86.7 c	90 a	96.7 a
Arosurf + ABG-6193	100 b	96.7 b	100 b	83.3 ab	76.7 b
Control	0 c	0 d	0 d	3.3 c	0 c

[a] Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Duncan's multiple range test [SAS Institute 1985]).

Table 2. Efficacy of water-based formulations of Arosurf® MSF and three preparations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) against 1st–4th instar larvae and pupae of *Anopheles albimanus* 48 hr posttreatment.

Water-based formulation	Cumulative mean percent mortality of each larval instar and pupal stage indicated[a]				
	1st	2nd	3rd	4th	Pupal
Arosurf MSF	26.7 a	16.7 a	26.7 a	83.3 ab	100 a
Teknar	100 b	100 b	100 b	86.7 ab	6.7 b
Bactimos	100 b	100 b	100 b	80.0 ab	0 b
ABG-6193	100 b	100 b	100 b	53.3 b	3.3 b
Arosurf + Teknar	100 b	100 b	100 b	100 a	100 a
Arosurf + Bactimos	100 b	93.7 c	100 b	100 a	100 a
Arosurf + ABG-6193	100 b	96.7 b	100 b	93.3 a	100 a
Control	0 c	0 d	0 c	3.3 c	0 b

[a] Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Duncan's multiple range test [SAS Institute 1985]).

Table 3. Efficacy of water-based formulations of Arosurf® MSF and three preparations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) against 1st–4th instar larvae and pupae of *Anopheles albimanus* 72 hr posttreatment.

Water-based formulation	Cumulative mean percent mortality of each larval instar and pupal stage indicated[a]				
	1st	2nd	3rd	4th	Pupal
Arosurf MSF	30.0 a	23.3 a	33.3 a	83.3 ab	100 a
Teknar	100 b	100 b	100 b	86.7 ab	6.7 b
Bactimos	100 b	100 b	100 b	80.0 ab	0
ABG-6193	100 b	100 b	100 b	53.3 b	3.3 b
Arosurf + Teknar	100 b	100 b	100 b	100 a	100 a
Arosurf + Bactimos	100 b	100 b	100 b	100 a	100 a
Arosurf + ABG-6193	100 b	100 b	100 b	100 a	100 a
Control	0 c	0 c	0 c	3.3 c	0 b

[a] Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Duncan's multiple range test [SAS Institute 1985]).

Table 4. Efficacy of water-based formulations of Arosurf® MSF and three preparations of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) in preventing eclosion of *Anopheles albimanus* eggs.

Water-based formulation	Cumulative mean percent of egg eclosion[a]
Arosurf MSF	25.3 a
Teknar	92.0 b
Bactimos	97.3 b
ABG-6193	94.7 b
Arosurf + Teknar	21.3 a
Arosurf + Bactimos	24.0 a
Arosurf + ABG-6193	24.0 a
Control	97.3 b

[a] Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Duncan's multiple range test [SAS Institute 1985]).

mained there and resulted in a low eclosion rate due to the film disruption of the air-water interface (Table 4).

All combined formulations produced over 90% mortality within all instars and pupae by 48 hr posttreatment (Table 2). With Arosurf MSF alone, mortality for pupae and 4th instar larvae was 100% and 83%, respectively by 24 hr, but less than 30% mortality occurred in 1st–3rd instars. The three *B.t.i.* formulations alone produced 100% mortality in 1st–3rd instar larvae by 48 hr posttreatment (Table 2) as expected. Teknar and Bactimos produced 86.7% and 80% mortality, respectively, in 4th instar larvae 24 hr posttreatment. This unusually high mortality of 4th instar larvae with formulations containing only *B.t.i.* was probably due to the use of early 4th instar larvae (less than 12 hr postmolt) which were still actively feeding.

All larvae treated with formulations containing Arosurf MSF dropped from their normal anopheline respiratory position at the surface to the bottom of the beaker where they formed an elliptic shape and were observed attempting to remove the film from their posterior spiracular apparatus with their mouthparts. Fourth instar larvae contorted repeatedly in attempts to restore contact with the air-water interface, but within an hour, dropped to the bottom, and died. First through 3rd instar larvae restored contact with the air-water interface in an abnormal vertical resting position, and presumably, obtained air in this manner.

Acute sensitivity by *An. albimanus* 4th instar larvae to Arosurf MSF formulations was observed at 24 hr (83% mortality), similar to that reported by Levy et al. (1982b) and White and Garrett (1977) for *An. quadrimaculatus* Say and *An. crucians* Wiedmann. First through third instar *An. albimanus* larvae were not as acutely sensitive to Arosurf MSF at 24 hr (16.7–20% mortality).

The observed difference in sensitivity between larval instars could be due to the morphological differences on the dorsal surface of the larvae and instar differences in reestablishment of air-water interface contact for respiration. Fourth instar *An. albimanus* larvae, typical of other *Anopheles* species, possess larger and more numerous dorsal setae compared with 1st–3rd instars. Thus, the difference in sensitivity and mortality to Arosurf MSF among 4th instars and earlier instars may be explained by the increased surface area on setae available for film deposition and the reduced surface tension of the air-water interface by the film.

Age structure of the immature stages had a pronounced effect on their sensitivity to all formulations tested. As anticipated, with formulations of *B.t.i.* alone, high mortality was found for 1st–3rd instars, with a decrease in the 4th larval instar and pupal stage mortality. This was due to the mode of action of *B.t.i.* as a gastric toxin which must be ingested by the mosquito larvae while feeding. The reverse occurred with Arosurf MSF formulations alone, with 4th instar

larvae and pupae being highly sensitive and earlier larval instars more tolerant. This finding was similar to that reported for *Cx. quinquefasciatus* by Levy et al. (1984).

In conclusion, Arosurf MSF alone is effective against eggs, pupae and 4th instar larvae of *An. albimanus*, but has limited effect on early larval instars. Early larval stages, although not directly affected by Arosurf MSF, never reached adulthood due to film persistence and the successive molting of the instars to susceptible stages. Arosurf MSF combined with any of the *B.t.i.* components tested was effective against all *An. albimanus* immature stages, and appears from this study to offer great potential as an alternative control strategy that is both effective against this important malaria vector and environmentally safe.

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