Am. J. Trop. Med. Hyg., 80(5), 2009, pp. 758–763

Efficacy of Aquatain™, a Monomolecular Surface Film, against the Malaria Vectors *Anopheles stephensi* and *An. gambiae* s.s. in the Laboratory

Tullu Bukhari and Bart G. J. Knols*

Laboratory of Entomology, Wageningen University and Research Centre, Wageningen, The Netherlands

Abstract. Monomolecular films are used for mosquito control because of their asphyxiating effect on larvae and pupae. Compared with other films, Aquatain mosquito formulation (AMF™) has an improved spreading ability and flexibility on a water surface. In the laboratory, AMF™ showed larvicidal, pupicidal, and oviposition deterrent effects against the malaria vectors *Anopheles stephensi* and *An. gambiae*. At a dose of 1 mL/m^2, the median lethal time to death for late larval instars was 3.02 (95% confidence interval [CI] = 2.76–3.25) and 0.98 (95% CI = 0.75–1.20) days for *An. stephensi* and *An. gambiae,* respectively. None of the treated larvae pupated. Pupal mortality reached 100% within two hours for both species. AMF™ repelled gravid females from ovipositing in treated oviposition cups. Without the choice of an untreated cup, the lowered water surface tension caused most females to drown while attempting to oviposit. This physical control method has the potential to become a safe, cost-effective, and resistance-proof malaria vector control tool.

INTRODUCTION

Monomolecular layers differ from other mosquito control agents because of their ability to target multiple stages in the mosquito life cycle.[1–5] All stages that come in contact with the water surface (e.g., eggs, larvae, pupae, emerging adults, and ovipositing females) are affected by the lowered surface tension caused by such layers.[5,6] As a result, these layers can provide the combined benefits of larval and adult control, which leads to reduction in mosquito density and longevity.[7–11]

Ethoxylated isostearyl alcohols are plant-derived monomolecular layers that have been rigorously tested in laboratory and field settings.[1,2,5,6] Commercially known as Arosurf® MSF (monomolecular surface film) and Agnique® MMF (monomolecular film), these layers were found to be efficient in killing pupae and late larval instars in addition to causing drowning of eggs and ovipositing females.[5] Their mixing with other larvicides (e.g., *Bacillus thuringiensis israelensis* [*Bti*], *B. sphaericus* [*Bs*], and diesel fuel) led to an effective control of the early larval stages.[4,5] Various studies showed these films to be environmentally friendly and suitable for a variety of habitats including marshes, pastures, water tanks, sewer systems, and tree holes.[1,5,12] However, these products could not be successfully incorporated into control programs because of their inability to withstand wind and the tendency to accumulate around debris and vegetation.[5] This disadvantage rendered them unfavorable for the treatment of large and vegetated habitats such as rice paddies and irrigation canals, which are known to harbor vector populations that may contribute substantially to malaria transmission.[13–16]

Aquatain™ is a monomolecular film that has been designed and successfully tested as an anti-evaporation liquid to prevent water loss from large water storage basins, e.g., dams in hot climates.[17] In contrast to Arosurf® MSF and Agnique® MMF, Aquatain™ is a polydimethylsiloxane (PDMS, 80%)–based liquid, which is characterized by its ability to cover large vegetated areas and resilience to wind and rain.[18] It is reported to have no adverse effect on the water quality and has recently been certified (certificate# 4Q360-01; NSF International, Ann Arbor, MI) for use on drinking water.[19,20] The spreading ability and flexibility provides Aquatain™ an advantage over other known monomolecular layers, and its mosquito control potential is worth testing.

In this study we tested the efficacy of Aquatain™ mosquito formulation (AMF™, Ultimate Agri-Products, Noble Park, Victoria, Australia) against *Anopheles stephensi* Liston and *An. gambiae* s.s. Giles, which are important malaria vectors in Asia and Africa, respectively. The difference between Aquatain™ and AMF™ is the addition of 2% eucalyptus oil in the latter compound. A comparison was also made between the efficacy of AMF™ and the original anti-evaporation formulation (Aquatain™) against the same species.

MATERIALS AND METHODS

Mosquitoes. *Anopheles stephensi* eggs (Strain STE 2, MRA no. 128, origin India) were obtained from the Malaria Research and Reference Reagents Resource Center, Centers for Disease Control and Prevention (Atlanta, GA), placed in water trays (30 × 15 × 7 cm), and kept in a climate-controlled chamber maintained at a temperature of 27 ± 1°C, 12L:12D photoperiod, and a relative humidity of 80 ± 5%. Larvae were fed on Liquifry No 1 (Interpet Ltd., Dorking, Surrey, United Kingdom) for the first three days and Tetramin® for the rest of their larval development. Pupae were collected and transferred in small cups to cages (30 × 30 × 30 cm) for emergence. Adults had *ad libitum* access to 6% glucose. Females, when 4–5 days old, were offered blood from the forearm of a volunteer, and an oviposition cup with wet filter paper was then placed inside the holding cage for egg collection. *An. gambiae* s.s., (Suakoko strain, courtesy of M. Coluzzi) were reared under similar conditions.

Experimental design. All experiments were conducted in a climate-controlled room at 27 ± 1°C, a 12D:12L photoperiod, and a relative humidity of 80 ± 5%. Tap water was kept in open plastic trays a day before every experiment to remove chlorine. Aquatain™ and AMF™ were used as recommended by the manufacturer. Eucalyptus oil provides an additional toxicity mode of action to AMF™ and is an oviposition repellent.[21,22]

Larvicidal effect. The larvicidal effect of AMF™ was tested against L_1–L_2 (young, 1–3 days) and L_3–L_4 (old, 4–8 days)

* Address correspondence to Bart G. J. Knols, Laboratory of Entomology, PO Box 8031, 6700 EH, Wageningen University and Research Centre, Wageningen, The Netherlands. E-mail: bart.knols@wur.nl

instars separately. Four plastic trays (13 × 15 × 7 cm) were filled with 1 liter of water and 50 larvae were added to each tray along with Tetramin®-water solution (0.2–0.3 mg/larva/tray/day). The surface area of water was 0.035 m^2. Using the recommended dose of 1 mL/m^2, 35 μL AMF™ was applied to one of the trays. Half (17.5 μL) and double (70 μL) the recommended dose was pipetted in a second and third tray. One tray remained untreated and served as the control. Mortality was checked daily and recorded for ten days. Three replicates were performed. Tetramin®-water solution was adjusted according to the daily mortality.

Pupicidal effect. Four plastic oviposition cups (5 cm diameter × 3.2 cm height) were filled with 40 mL of water. Fifteen pupae were added to each cup. Surface area was calculated to be 0.002 m^2. On the basis of the recommended dose, 2 μL of AMF™ was required. A total of 2 μL, 1.0 μL, and 0.5 μL was pipetted into three cups. A fourth cup was not treated and served as the control. The number of dead pupae was counted after every 15 minutes for 2 hours. Four replicates were performed. A pupa was considered dead if it did not show the characteristic stretching reaction on slight dipping.

Effect on oviposition. Female mosquitoes, 4–8 days old, were blood fed. For *An. gambiae*, females were blood fed twice. After 2 days, 12 gravid females were transferred to a cage that contained a 6% glucose water bottle and two oviposition cups (similar to those described above). One cup was treated with 2 μL of AMF™ (recommended dose) and the other served as the control. The cups were placed at two opposite corners of the cage (30 × 30 × 30 cm) and their positions were switched between replicates to avoid positional effects. After 48 hours, the eggs laid in each oviposition cup were counted. Four replicates were performed.

In the second experiment, the females were placed in a no-choice situation. In this case, each cage contained only one oviposition cup (as described above), a glucose water bottle and 12 gravid females. Treatments (in separate cages) included 2 μL 1.0 μL, and 0.5 μL of AMF™ and the control. Three replicates were performed. On the second day, the number of eggs laid were counted and all the females were dissected to count the number of eggs that had developed up to Christopher IV and IV–V transition stages but had not been laid.[23] The unlaid eggs were taken into account to be sure that on average the potential for laying eggs was the same in each cage.

Effect of eucalyptus oil. To compare AMF™ and Aquatain™ for larvicidal and pupicidal effects, bioassays were performed with L_3–L_4 larvae and pupae. Treatments included AMF™ (with eucalyptus oil) and Aquatain™ (no eucalyptus oil). The minimal dose was used (17.5 μL for larvae and 0.5 μL for pupae), to pronounce any difference in effect in the same settings as described above. Three replicates were performed. Aquatain™ was also tested in the choice and no-choice oviposition experiments. In case of the choice experiments (four replicates), the gravid females had a choice between an untreated and Aquatain™-treated oviposition cup. In the no-choice (three replicates) experiment, gravid females had access to either an untreated, AMF™-treated, or Aquatain™-treated cup. AMF™ or Aquatain™ was applied at the lowest dose (0.5 μL).

Statistical analysis. In case of more than 10% mortality in the control, the percentage mortality data for the corresponding treatments was corrected for natural mortality by Abbott's formula to calculate median lethal time to death (LT_{50}) values by probit analysis.[24,25] The effect of species, larval stage, and treatments was analyzed using Cox regression and Kaplan-Meier pairwise comparisons.[26] A one-way analysis of variance was used to detect significant differences between the number of eggs laid in each cage. All analyses were performed using SPSS version 15 software (SPSS Inc., Chicago, IL).

RESULTS

Larvicidal effect. For all treatment doses, a minimum of 69% mortality was observed in the 10-day period after exposure (Figure 1). The effect on larval stages was significant. For both species, the LT_{50} values for the L_1–L_2 larvae were higher than those for L_3–L_4 larvae at corresponding doses (Table 1). The hazard ratio (HR) (95% confidence interval [CI]) for the L_3–L_4 stage was 3.6 (3.14–4.20) and 1.9 (1.74–2.28) times that for L_1–L_2 stage of *An. gambiae* and *An. stephensi*, respectively. This difference was also observed by pairwise comparison between survival curves of both stages ($P < 0.001$ for each species). Cox regression showed no increase in the HR for the larvae of *An. stephensi* at the L_1–L_2 stage compared with *An. gambiae* except for the dose of 17.5 μL (HR = 2.2, 95% CI = 1.4–3.5) but a significant increased HR at the L_3–L_4 stage for all treatments. At the L_3–L_4 stage, the HR (95% CI) for *An. gambiae* was 9.8 (4.0–23.7), 3.1 (1.2–7.5), and 7.4 (3.0–17.8) for the 17.5-μL, 35-μL and 70-μL treatments, respectively, compared with *An. stephensi*. All treatments were significantly different from the controls ($P < 0.001$) but an increase in concentration did not show a systematic increase in mortality because the survival curves for various treatments did not differ in any specific pattern (Figure 1). None of the larva in any of the treatments, apart from the control, molted into a pupa (Table 1).

Pupicidal effect. For pupae, the species effect was more pronounced (Figure 2). The HR (95% CI) for *An. gambiae* pupae was 6.9 (4.5–10.5) times that for *An. stephensi*. The species effect is also apparent from the LT_{50} values (Table 2). For *An. gambiae*, no significant difference was found between the different doses tested. For *An. stephensi*, however, the 1-μL treatment was significantly ($P < 0.05$) more effective than the other treatments. Although none of the pupae for both species at all concentrations did so, all of the pupae in the control treatment emerged into adults.

Effect on oviposition. In the choice experiment, gravid females had an option of laying eggs in an AMF™-treated cup and/or an untreated oviposition cup. Both species did not lay any eggs in the treated cups. A mean ± SE of 378 ± 91 eggs and 227 ± 39 eggs were laid in the control cups by *An. stephensi* and *An. gambiae*, respectively (Table 3).

In the no-choice experiments, gravid females were provided with a single cup that was either untreated or treated with one of the concentrations (0.5 μL, 1.0 μL, or 2.0 μL). Most females drowned in treated cups in an attempt to oviposit; only one *An. gambiae* drowned in a control cup. In contrast to complete absence of oviposition in the treated cups, a mean ± SE of 567 ± 126 and 217 ± 86 eggs were laid in the control cages by *An. stephensi* and *An. gambiae*, respectively. All females were dissected and eggs developed up to Christopher IV and IV–V transition stages were counted. There was no significant difference in the total number of eggs, laid and unlaid, in any treatment cage for *An. stephensi* (F = 3.482, degrees of

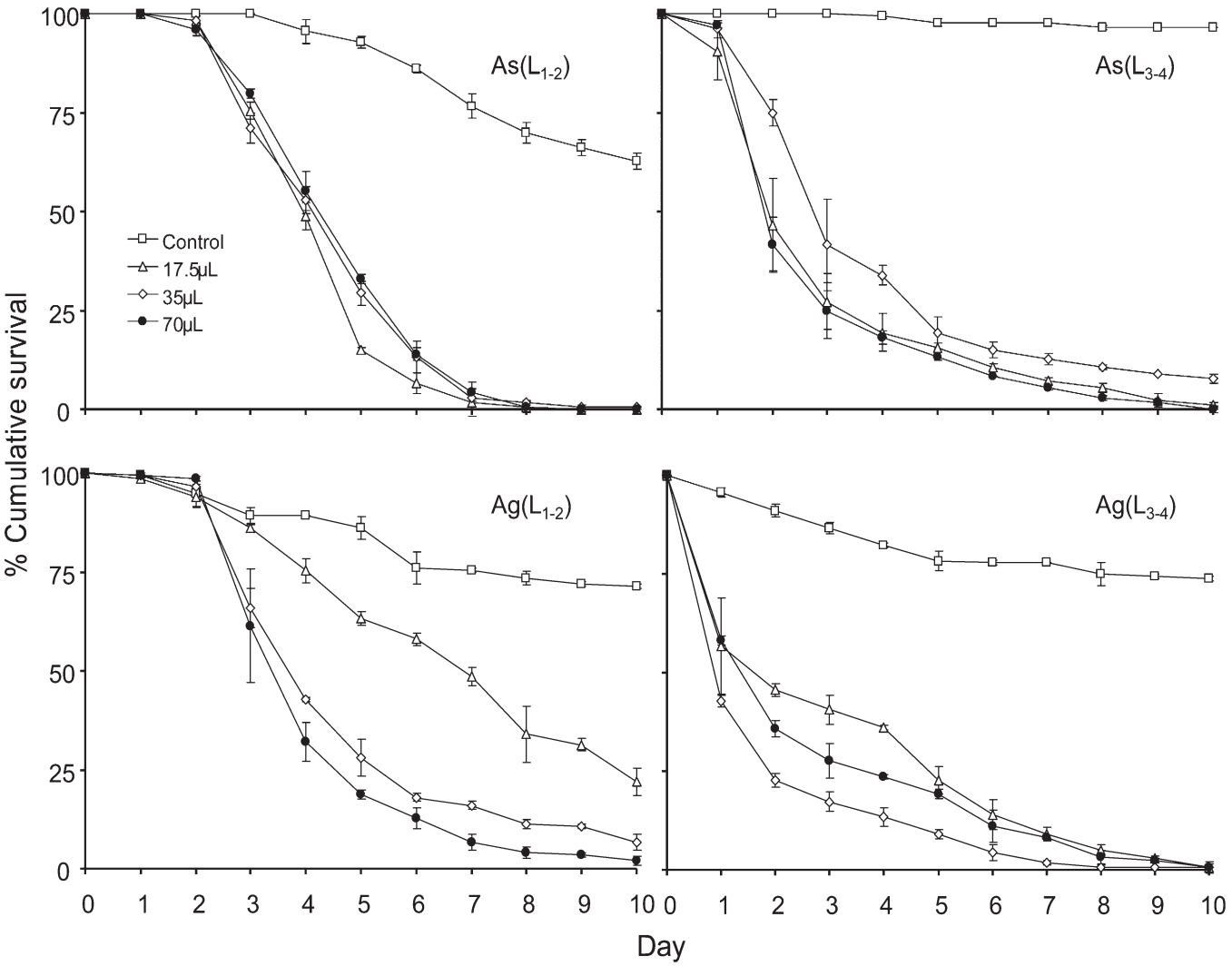

FIGURE 1. Cumulative percentage survival for early (L_{1-2}) and late (L_{3-4}) larval instars of *Anopheles stephensi* (As) and *An. gambiae* (Ag) in untreated (control) and Aquatain™-treated (17.5 µL, 35 µL, and 70 µL) larval trays. Percentages were not corrected for natural mortality.

freedom [df] = 3, 8, $P > 0.05$) and *An. gambiae* (F = 0.295, df = 3, 8, $P > 0.05$).

Effect of eucalyptus oil. No additional effect of eucalyptus oil was found for larvae or pupae in terms of mortality when directly compared with Aquatain™. However, in case of oviposition, unlike AMF™, in the choice experiments a mean ± SE of 1.5 ± 0.6 females of *An. stephensi* and 8.3 ± 1.3 females of *An. gambiae* drowned while attempting to oviposit, although they had the choice of an untreated oviposition cup (Table 3). This finding shows that Aquatain™ by itself does not repel the gravid females. Another difference was that a mean ± SE of 11.8 ± 7.8 eggs were laid in the treated cups; egg laying was not observed with AMF™ treatment.

In the no-choice experiment (Table 4), no eggs were found in AMF™-treated cups and only 27 eggs were found in one of the cups treated with Aquatain™. In the control, a mean ± SE of 233.0 ± 34.9 and 178.7 ± 57.5 eggs were laid by *An. stephensi* and *An. gambiae*, respectively. No significant difference was found in the total number of eggs per cage when the dissected eggs were also taken into account for *An. stephensi* (F = 0.889, df = 2, 6, $P > 0.05$) and *An. gambiae* (F = 0.303, df = 2, 6, $P > 0.05$).

DISCUSSION

Our results show the efficacy of Aquatain™ against multiple life cycle stages of *An. stephensi* and *An. gambiae* in the laboratory. In the larval experiments, the L_3–L_4 stage was more susceptible to AMF™ than the L_1–L_2 stage. The same response was reported by Das and others[2] for *An. stephensi* when treated with Arosurf® MSF. The late stages are affected more because of their reduced ability to use dissolved oxygen.[2,21,27] The failure of larvae to pupate in any of the treated trays was a persistent result. A possible reason may be the reduced larval fitness because they showed stunted growth and had a loss of appetite, which was apparent from the accumulation of food in the treated trays. Corbet and others[21] showed that larvae treated with PDMS spend more time on the surface; this behavioral change affects their fitness. The pupal stage showed a more drastic effect of AMF™ because they lack the ability to use dissolved oxygen completely. Arosurf® MSF also affected the pupae more than the larvae.[2]

The unsystematic effect of different concentrations on the mortality of larvae and pupae may be caused by the mode of action. Polydimethylsiloxane is a bimodal agent. It changes

TABLE 1

Median lethal time to death (LT_{50}, in days) with 95% confidence intervals (CIs) for young (L_1–L_2) and old (L_3–L_4) larvae of *Anopheles stephensi* and *An. gambiae* (n = 150) after exposure to various doses of AMF™*

Species	Stage	Dose (µL)	Slope + SE	LT_{50} (95% CI)†	χ^2	% Mortality	% Pupation
An. stephensi	L_1–L_2	0	1.737 + 0.18	NA[a]	3.2	38.9	61
		17.5	3.284 + 0.20	3.83 (3.6–3.9)[b]	3.5	99.3	0
		35.0	2.729 + 0.15	4.01 (3.8–4.2)[c]	7.8	99.1	0
		70.0	2.872 + 0.16	4.13 (3.9–4.3)[c]	9.0	100.0	0
	L_3–L_4	0	0.787 + 0.28	NA[a]	1.9	3.3	96
		17.5	1.331 + 0.08	2.16 (1.9–2.4)[b]	9.4	98.9	0
		35.0	1.356 + 0.08	3.02 (2.7–3.2)[c]	9.4	91.9	0
		70.0	1.531 + 0.09	2.19 (1.8–2.5)[d]	21.5	100.0	0
An. gambiae	L_1–L_2	0	0.731 + 0.09	NA[a]	3.5	28.6	53
		17.5	1.633 + 0.13	8.01 (7.5–8.6)[b]	20.7	69.7	0
		35.0	1.603 + 0.11	4.15 (3.8–4.4)[b,c]	7.0	90.7	0
		70.0	2.017 + 0.14	3.60 (3.3–3.8)[c]	4.6	97.2	0
	L_3–L_4	0	0.451 + 0.07	NA[a]	1.4	26.1	72
		17.5	0.878 + 0.06	2.01(1.1–2.7)[b,d]‡	45.6	99.2	0
		35.0	0.891 + 0.07	0.98 (0.7–1.2)[c]	12.4	99.2	0
		70.0	0.880 + 0.06	1.60 (1.3–1.8)[d]	19.4	99.3	0

*NA = not applicable.
†Values without letters in common differ ($P < 0.05$) with respect to survival curves.
‡A heterogeneity factor was used in the calculation of the CLs.

the surface tension of the water and floods the respiratory organs, which results in the tail-nibbling behavior observed. The flooding feature is more dominant.[21] All larvae on the surface at the time of treatment are likely to be instantly effected because of the flooding feature. As long as the amount of AMF™ is enough to flood the trachea of the larvae, further increase in the concentration of AMF™ probably has no additional effect.

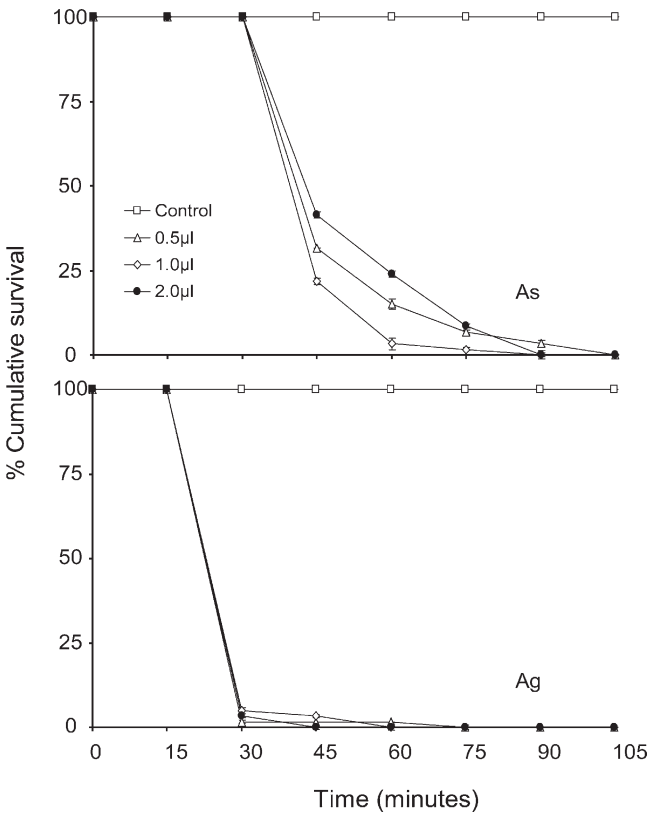

FIGURE 2. Cumulative percentage survival for pupae of *Anopheles stephensi* (As) and *An. gambiae* (Ag) in untreated (control) and Aquatain™-treated (0.5 µL, 1.0 µL, and 2.0 µL) oviposition cups.

Aquatain™ and AMF™ showed no difference in their effect on larvae and pupae, which suggests that Aquatain™ without the addition of a repellent has an equal potency to act as a control agent. Eucalyptus oil has a concentration-dependent oviposition repellent effect, which is why in the choice experiment with AMF™ the females were repelled from the treated cups.[22] However if gravid females are not repelled, as in case of Aquatain™, it rules out the chance that the females will search for an alternative site to lay eggs. This non-repellent effect is useful because it provides an increased chance of drowning a gravid female. It will thus be preferable to apply Aquatain™ for mosquito control.

The difference in the mean ± SE number of *An. stephensi* (1.5 ± 0.64) and *An. gambiae* (8.25 ± 1.31) females that drowned in the Aquatain™ choice experiment (Table 3) is probably linked to a difference in their oviposition behavior. Gravid females of *An. gambiae* are thought to oviposit eggs during flight or by sitting on the water surface, but not when vertically perched.[28] Conversely, *An. stephensi* females were observed to cling to the brim of the oviposition cup with their fore tarsi while ovipositing. As a result, *An. gambiae* females were unable to avoid drowning once they landed on the treated surface whereas *An. stephensi* females could pull themselves out.

In addition to these results, Aquatain™ has certain properties that indicate the advantages that will be associated with using it as a control agent. Aquatain™, unlike contemporary control agents, has a physical mode of action. It is therefore less likely that resistance will develop against it. Other monomolecular

TABLE 2

Median lethal time to death (LT_{50}, in minutes) with 95% confidence intervals (CIs) for various doses of AMF™ treatment of *Anopheles stephensi* (n = 60) and *An. gambiae* pupae (n = 60)*

Species	Dose (µL)	Slope + SE	LT_{50} (95% CI)†	χ^2
An. stephensi	0.5	3.41 + 0.20	45.8 (44.0–47.5)[a]	23.3
	1	4.08 + 0.26	44.0 (42.4–45.5)[b]	25.0
	2	3.29 + 0.19	47.5 (45.6–49.3)[a]	17.5
An. gambiae	0.5	4.28 + 0.47	20.4 (19.2–21.5)[a]	13.4
	1	4.86 + 0.62	20.4 (19.3–21.3)[a]	0.10
	2	4.76 + 0.62	20.0 (18.9–20.9)[a]	0.13

*All treatments resulted in 100% mortality within 2 hours.
†Values without letters in common differ ($P < 0.05$) with respect to survival curves.

TABLE 3

Number of eggs laid and females (n = 12) that drowned during various treatments during the choice oviposition experiment in which gravid *Anopheles stephensi* and *An. gambiae* females had access to a treated and an untreated oviposition cup

Species	Treatment	Cup	Eggs laid, mean ± SE	No. that drowned, mean ± SE
An. stephensi	AMF™	Treated	0	0
		Untreated	377.6 ± 91.1	0
	Aquatain™	Treated	0	1.5 ± 0.64
		Untreated	265.5 ± 23.7	0
An. gambiae	AMF™	Treated	0	0
		Untreated	226.6 ± 38.9	0.25 ± 0.12
	Aquatain™	Treated	11.7 ± 7.81	8.25 ± 1.31
		Untreated	87.0 ± 22.2	0

surface films such as Arosurf® MSF and Agnique® MMF have the same mode of action and resistance has not been reported thus far. Owing to its safety and non-toxicity, Aquatain™ will be suitable for all kinds of breeding sites and no personal protection will be required during application.[19,20] The self-spreading feature will make it easier to apply Aquatain™ over large and otherwise inaccessible areas.[29] This feature will be particularly useful in controlling larvae and pupae of the African vector *An. funestus*, which occupies larger, more permanent sites.[13] An additional advantage in applying Aquatain™ will be the reduced water evaporation as the breeding sites are often economically and domestically important.[30]

Surface films are more effective at the late larval and pupal stage in contrast to *Bti* and *Bs*.[5] Because application of *Bti* and *Bs* has been found to be cost-effective, we compared their cost with Aquatain™ when applied on a hectare of breeding site for a year.[31] Considering the fact that Aquatain™ applied at a dose of 0.5 mL/m^2 caused 100% pupal mortality within two hours and ensured no pupation in the larval trays, we based our calculations on this concentration. Furthermore, we assumed that once a breeding site is treated, the film remains effective for 10 days (according to supplier specifications). On the tenth day, if a female successfully lays eggs, the resulting larvae need at least seven days before developing into pupae. Therefore, subsequent treatment can be delayed for an additional seven days.

The cost of Aquatain™ at a rate of 0.5 mL/m^2 per hectare after every 17 days for a year is $1,890 ($18 per liter), which is comparable to the water-dispersable granule ($1,300–1,825) and commercial corn granules ($1,466–1,955) formulations of *Bs* if applied after every 14 days per hectare per year.[31] The cost of similar formulations of *Bti* are $208–313 and $563–782 for the water-dispersable granule and commercial corn granules formulations, respectively, but this estimate does not include the labor cost involved with repetitive application. The required application frequency of Aquatain™ is less than half that for *Bti*. However, because these estimates do not consider untreated periods that can be incorporated without compromising efficiency of a control program, actual costs would be lower than these estimates. We are working on various formulations of Aquatain™ that can further reduce the application frequency to approximately once a month in permanent breeding sites. Another logistic advantage of Aquatain™ will be the shelf life, which for various formulations of *Bs* and *Bti* is 2–3 years when stored at a temperature below 25°C. High temperature reduces their efficacy and must be considered during transportation.[32,33] Aquatain™ has no such limitations. It has been reported to remain stable after being kept at 54°C for 2 weeks.[34]

The laboratory results, properties, and comparison with well-tested larvicides suggest that Aquatain™ is a promising control agent. However, for a more realistic overview, field trials need to be conducted. Field trials will also provide an opportunity to detect any non-target effects; although none have been reported so far when Aquatain™ was used in Australia.[19] In the event of the expected field results, Aquatain™ may be incorporated, as a new control tool, into integrated mosquito control programs.

TABLE 4

No-choice oviposition experiment testing the additional effect of eucalyptus oil on the egg laying of *Anopheles stephensi* and *An. gambiae**

Species	Treatment	Eggs		No. that drowned
		Laid + dissected†	Laid	
An. stephensi	Control	251 ± 51.9^a	233 ± 34.9	0
	AMF™	200 ± 53.2^a	0	7.0 ± 2.0
	Aquatain™	171 ± 1.52^a	9 ± 9	6.3 ± 0.9
An. gambiae	Control	338 ± 98.8^a	179 ± 57.5	0
	AMF™	286 ± 29.8^a	0	11.0 ± 0.5
	Aquatain™	371 ± 87.7^a	0	10.3 ± 0.9

*Values are the mean ± SE number of eggs (laid + dissected), eggs laid, and females (n = 12) that drowned in control or treated oviposition cups.
†Values without letters in common differ significantly at $P < 0.05$.

Received November 20, 2008. Accepted for publication February 13, 2009.

Acknowledgments: We thank David Coe and Graham Strachan (Ultimate Agri-Products, Noble Park, Victoria, Australia) for providing AMF™ and its specifications; the Malaria Research and Reference Reagents Resource Center, Centers for Disease Control and Prevention (Atlanta, GA) for providing *An. stephensi* eggs; Leo Koopman, André Gidding, and Frans van Aggelen for rearing *An. gambiae* mosquitoes; Dr. Sander Koendraadt for helping with statistical analysis; and Dr. Ulrike Fillinger for comments on the on the initial draft of the manuscript; and three anonymous reviewers for useful comments.

Financial support: Tullu Bukhari is supported by the Higher Education Commission, Pakistan through The Netherlands Organization for International Cooperation in Higher Education. Bart G. J. Knols is supported by a VIDI grant (#864.03.004) from the Dutch Scientific Organization.

Authors' addresses: Tullu Bukhari and Bart G. J. Knols, Laboratory of Entomology, PO Box 8031, 6700 EH, Wageningen University and Research Centre, Wageningen, The Netherlands, E-mails: tullu.bukhari@wur.nl and bart.knols@wur.nl.

REFERENCES

1. Batra CP, Mittal PK, Adak T, Subbarao SK, 2006. Efficacy of Agnique®; MMF monomolecular surface film against *Anopheles stephensi* breeding in urban habitats in India. *J Am Mosq Control Assoc 22:* 426–432.
2. Das PK, Tyagi BK, Somachari N, Venkatesan V, 1986. Efficacy of Arosurf a monomolecular surface film in controlling *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti. Indian J Med Res 83:* 271–276.
3. Levy R, Chizzonite JJ, Garrett WD, Miller TW, 1982. Efficacy of the organic-surface film isostearyl alcohol containing 2 oxyethylene groups for control of *Culex* and *Psorophora* mosquitos—laboratory and field studies. *Mosq News 42:* 1–11.
4. Levy R, Putnam JL, Miller TW Jr, 1986. Laboratory evaluations of formulations of Arosurf MSF and *Bacillus sphaericus* against larvae and pupae of *Culex quinquefasciatus. J Am Mosq Control Assoc 2:* 233–236.

5. Nayar JK, Ali A, 2003. A review of monomolecular surface films as larvicides and pupicides of mosquitoes. *J Vector Ecol 28:* 190–199.
6. Service M, 2008. *Medical Entomology for Student*s. Cambridge, United Kingdom: Cambridge University Press.
7. Killeen GF, 2003. Following in Soper's footsteps: northeast Brazil 63 years after eradication of *Anopheles gambiae*. *Lancet Infect Dis 3:* 663–666.
8. Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BGJ, 2002. Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa. *Lancet Infect Dis 2:* 618–627.
9. Killeen GF, Fillinger U, Knols BGJ, 2002. Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malar J 1:* 8.
10. Macdonald G, 1957. *The Epidemiology and Control of Malaria*. Oxford, United Kingdom: Oxford University Press.
11. White GB, 1999. Malaria prevention by vector control: effectiveness of insecticidal methods. *Parassitologia 41:* 385–387.
12. Karanja DM, Githeko AK, Vulule JM, 1994. Small-scale field evaluation of the monomolecular surface film 'Arosurf MSF' against *Anopheles arabiensis* Patton. *Acta Trop 56:* 365–369.
13. Dolo G, Briët OJT, Dao A, Traoré SF, Bouaré M, Sogoba N, Niaré O, Bagayogo M, Sangaré D, Teuscher T, Touré YT, 2004. Malaria transmission in relation to rice cultivation in the irrigated Sahel of Mali. *Acta Trop 89:* 147–159.
14. Keiser J, De Castro MC, Maltese MF, Bos R, Tanner M, Singer BH, Utzinger J, 2005. Effect of irrigation and large dams on the burden of malaria on a global and regional scale. *Am J Trop Med Hyg 72:* 392–406.
15. Ng'ang'a P, Shililu JI, Jayasinghe G, Kimani V, Kabutha C, Kabuage L, Kabiru E, Githure J, Mutero C, 2008. Malaria vector control practices in an irrigated rice agro-ecosystem in central Kenya and implications for malaria control. *Malar J 7:* 146.
16. Ijumba JN, Lindsay SW, 2001. Impact of irrigation on malaria in Africa: paddies paradox. *Med Vet Entomol 15:* 1–11.
17. Ultimate Agri-products, 2007. *Aquatain Independent Trial Results*. Available at: http://www.aquatain.com.au. Accessed November 20, 2008.
18. Ultimate Agri-Products. Available at: http://www.aquatain.com.au.
19. Ultimate Agri-products, 2007. *Aquatain Independent Water Quality Report*. Available at: http://www.aquatain.com.au. Accessed November 20, 2008.
20. International NSF, 2008. *Certificate for Use on Drinking Water in USA*. Available at: http://www.nsf.org/Certified/PwsChemicals/Listings.asp?Company=4Q360&Standard=060&. Accessed November 20, 2008.
21. Corbet SA, Tiley C, Moorhouse T, Giam C, Pursglove S, Raby J, Rich M, 2000. Surface films as mosquito larvicides: partitioning the mode of action. *Entomol Exp Appl 94:* 295–307.
22. Senthil NS, 2007. The use of *Eucalyptus tereticornis* Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Bioresour Technol 98:* 1856–1860.
23. World Health Organization, 1975. *Manual of Practical Entomology in Malaria, Part II*. Geneva: World Health Organization.
24. Abbott WS, 1925. A method for computing the effectiveness of an insecticide. *J Econ Entomol 18:* 265–267.
25. Finney DJ, 1971. *Probit Analysis*. Cambridge, United Kingdom: Cambridge University Press.
26. Cox DR, 1972. Regression models and life tables (with discussion). *J R Stat Soc B 34:* 187–220.
27. Clements AN, 1992. *The Biology of Mosquitoes. Development, Nutrition and Reproduction*. London: Chapman and Hall.
28. Clements AN, 1999. *The Biology of Mosquitoes. Sensory Reception and Behavior*. New York: CABI Publishing.
29. Fillinger U, Kannady K, William G, Vanek M, Dongus S, Nyika D, Geissbuhler Y, Chaki P, Govella N, Mathenge E, Singer B, Mshinda H, Lindsay S, Tanner M, Mtasiwa D, de Castro M, Killeen G, 2008. A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. *Malar J 7:* 20.
30. Mutuku FM, Alaii JA, Bayoh MN, Gimnig JE, Vulule JM, Walker ED, Kabiru E, Hawley WA, 2006. Distribution, description, and local knowledge of larval habitats of *Anopheles gambiae* s.l. in a village in western Kenya. *Am J Trop Med Hyg 74:* 44–53.
31. Fillinger U, Lindsay SW, 2006. Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Trop Med Int Health 11:* 1629–1642.
32. Couch TL, 2000. Industrial fermentation and formulation of entomopathogenic bacteria. *Entomopathogenic Bacteria: From Laboratory to Field Application*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 297–316.
33. Powell KA, Jutsum AR, 1993. Technical and commercial aspects of biocontrol products. *Pestic Sci 37:* 315–321.
34. Ultimate Agri-Products, 2008. *Evaluation of the Stability of the Physical Properties of Aquatain AMF Mosquito Control Product following Accelerated Storage Stability*. Agrisearch Analytical Pty Ltd. Available at: http://www.aquatain.com.au. Accessed November 20, 2008.