EFFICACY OF AGNIQUE® (MMF) MONOMOLECULAR SURFACE FILM AGAINST IMMATURE STAGES OF *ANOPHELES ARABIENSIS* PATTON AND *CULEX* SPP (DIPTERA: CULICIDAE) IN KHARTOUM, SUDAN

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Abstract. The efficacy of the larvicidal and pupicidal agent (Agnique®) MMF was evaluated against larvae of *An. arabiensis* and *Culex* (Diptera: Culicidae) under field conditions in Bahary Locality, Khartoum, Sudan. At an applied dosage of 0.25 ml/m^2, MMF resulted in 89.4, 79.8 and 88.2% reductions in L_3-L_4 instars *An. arabiensis* and 63.5% in *Culex* larvae (all stages) 24 to 72 hours post-treatment. Pupae were completely eliminated (100%) within 24 hours post-treatment. The earlier instars (L_1-L_2) of *An. arabiensis* were more tolerant with a 62.5% reduction at 72 hours post-treatment compared to (L_3-L_4) instars and pupae. At 7-days post-treatment Agnique® gave a 57.5% reduction in L_1-L_2 and 92.6% in L_3-L_4 instar larvae of *An. arabiensis* and 57.3% and 86.4% in *Culex* larvae and pupae, respectively. We conclude that Agnique® can perform effectively against L_3-L_4 instars and pupae of *An. arabiensis* for only 1 week, and 3 to 4 days against L_1-L_2 instars of *Culex* spp.

INTRODUCTION

Malaria is by far the world's most prevalent vector-borne disease; it exists in more than 107 countries, especially in Africa, Asia and South and Central America (WHO/UNICEF, 2005). Annually, 300-500 million malaria cases and 1.7-2.5 million deaths are recorded worldwide, where 80% of all cases and 90% of deaths are in sub-Saharan Africa (WHO/UNICEF, 2005).

Sudan, as one of these countries, has an annual estimated 7.5 million malaria cases and 35,000 deaths. This represents 50% and 70% of all EMRO cases and deaths, respectively (Malik *et al*, 2003). In Khartoum State (the capital), malaria is a major public health and political concern, where the whole state population (>5.3 million) are at risk for malaria infection (El Sayed *et al*, 2000). Annually, about 20.8% of outpatient and 13% of inpatient mortality are due to malaria (KSMOH, 2006). *Anopheles arabiensis* is the sole malaria vector (Petrarca *et al,* 2002).

Malaria transmission control by suppressing the adult mosquito vector (*ie*, IRS) is the most effective, but development of vector resistance to insecticides has limited their use (Killeen *et al*, 2002). Vector control measures targeting larval stages affect adult vector densities, thus limiting malaria transmission (Carter *et al*, 2000).

Since the 1990s, the Malaria Control Program in Khartoum State has relied on the implementation of larvicides, source reduction (intermittent irrigation), supplemented with ULV/Fogging and limited IRS. As the state intensifies its efforts to meet the expectations

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of the WHO Roll Back Malaria program, it has become of critical importance to develop and evaluate cost-effective and environmentally safe larvicidals as alternatives to temephos to prevent and/or delay the development of resistance.

This has led to research in non-insecticide compounds, particularly Agnique® MMF monomolecular surface film. Several studies have shown the monomolecular surface to be effective against different mosquito species in a variety of breeding habitats (White and Garrett, 1977; Levy *et al,* 1982b; Mulla *et al,* 1983; Takahashi *et al,* 1984; Karanja *et al,* 1994; Batra *et al,* 2006).

Nayar and Ali (2003) reviewed numerous published works on Arosurf® MSF and Agnique® MMF and concluded that both products, at different applied rates have relatively fewer mortality effects against first and second instar larvae of most *Aedes*, *Culex* and some anopheline species compared to third and fourth instars and pupae. The fourth instar larvae of most mosquito species are killed within 24 to 96 hours, while the pupae are killed within 24 hours of treatment. The present study was carried out to evaluate the efficacy of Agnique® MMF on the larvae and pupae of *An. arabiensis* and *Culex* spp.

MATERIALS AND METHODS

Study area (Fig 1)

The State of Khartoum is located in the middle of Sudan, at the junction of the White Nile and Blue Nile rivers between 15º 10′ and 16º 30′N latitude and 31º35′ and 40º 20′E longitude. It is the capital, political, and commercial center of the country. The state covers 28,000 km^2 with a total population of 5.352 million individuals.

The state is a semi-desert area with 3 seasons. The dry season, usually starts in March and ends in June with a mean temperature of 38ºC and a maximum peak of 45ºC in May, and a relative humidity of 20-40%. The rainy season extends from July to October, with a mean annual rainfall of 150 mm, and a maximum peak of 200 mm in August. The temperature range is 28º-35ºC, with a relative humidity of 40-60%. The winter season starts in November and ends in February with average maximum and minimum temperatures of 25ºC and 16ºC, respectively, and a relative humidity of 30-40%.

Ninety-seven point eight percent of Khartoum is considered cultivatable, under both irrigation and rain fed agriculture. Most of the land under irrigation in greater Khartoum is located in Bahary, Sharag el Neil, and in Khartoum localities. Vegetables, animals fodder, wheat, maize, sorghum, millet, sweet potatoes, beans, onion, groundnuts and fruits are produced.

Agnique® MMF, (ISA-2OE, ethoxlated isostearyl alcohol) developed by Cognis Corporation (Cincinnati, OH, USA and supplied by USM), was tested for the control of *An. arabiensis* larvae in Bahary, Khartoum, Sudan. Each pond size was 50 cm x 50 cm x 35 cm. The ponds were flooded with well water on October 23 and by October 26 almost all the

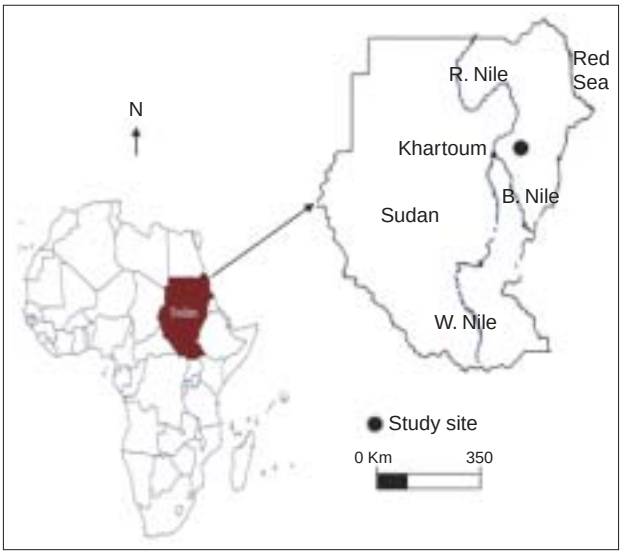
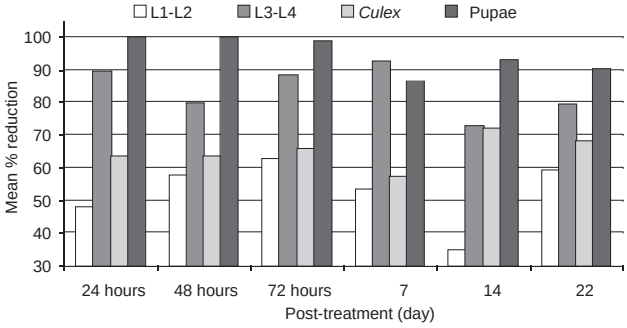


Fig 1–Map of Sudan showing the study site.



L_1-L_2: indicates first and second instars; L_3-L_4: indicates third and fourth instars.

% reduction: mean for three experiments.

Fig 2–Mean percent mortality of *An. arabiensis* (L_1-L_2 and L_3-L_4), *Culex* and pupae in ponds treated with MMF (0.25ml/m^2), at Bahary, Khartoum, Sudan, 3 November 2006 to 11 January 2007.

ponds became infested with anopheline and culicine larvae. Of the 10 ponds, 5 were treated with Agnique® and 5 served as untreated controls. The mean water temperature during the study period ranged from a low of 15.4ºC (8 to 26ºC) to high of 25.6ºC (15 to 34ºC) whereas the pH averaged 8.01 (7.8-8.2).

Treatment

On November 4, 2006, Agnique® (MMF) was applied at a concentration of 0.25 ml/m^2. The required amount was measured with a 5-ml syringe and applied at one point in each pond. The material spread rapidly, forming an invisible thin film on the water surface. The experiment (22 day monitoring period) was repeated three times, each time with two treatments, first in the beginning and the second cycle on Day13.

Larval sampling

Larval collection was made by the dipping method (Service, 1971) using a 350-ml dipper. Ten dips were recorded, at 0 (pre-treatment), 24, 48, 72 hours and Days7, 10, 12, 14, 16, 18, 20 and 22. *Anopheles* species larvae were categorized as (L_1-L_2 or L_3-L_4). The Mulla *et al* (1971) formula was used to calculate the percentage reduction (%) (see below). The maximum and minimum water temperatures of each pond were recorded.

% Reduction = 100 - [(C1/ T1) x (C2/T2)] x 100

C1 and T1 are the pre-treatment immature densities in the control and treatment ponds, and C2 and T2 are the post-treatment immature densities in the control and treatment ponds

Data analysis

The data was analyzed using SPSS for Windows, version 11.5. The average numbers of immature stages collected pre- and post-treatment in the treated and control ponds were analyzed for differences using nonparametric testing (Mann-Whitney *U* test) where appropriate. To determine the effectiveness of MMF in the field, the percent reduction in each pond was calculated as follows: Overall average pre-treatment larvae/dip from the control ponds was used as C1. The overall average pre-treatment larvae/dip for each treatment was used as T1. For each sample date, the mean larvae/dip for each control pond was used as C2 and the individual treatment pond average was used as T2. The overall mean treatment percent reduction was determined from the ponds percent reduction. The calculated pond percent reduction values less than zero were treated as zero for determination of the mean percent reduction. To determine the difference in percent larval reduction after treatment, time factors data were analyzed as described above. A p-value of <0.05 was statistically significant ($p<0.05$).

RESULTS

The study was carried out during the winter (3 November 2006 to 11 January 2007). Throughout the study period the mean water temperature ranged from a low of 15.4ºC (range 8-26ºC) and a high of 25.6ºC (range 15-34ºC).

During the sixty-six day testing period, 22,519 immature stage mosquito species were collected. *Anopheles arabiensis* was the most common species throughout the study period. It constituted 68.2% (*n*= 15,354) of the total, whereas the remaining were *Culex* larvae in 24.7% (*n*= 5,561) and mixed pupal stages for both *An. arabiensis* and *Culex* species in 7.1% (*n*=1,604).

The results of the Mann-Whitney *U* test showed that the overall densities of *Anopheles* and *Culex* larvae and pupae were significantly different L_1-L_2 larvae of *An. arabiensis* (*U*=9387.5; p<0.001) L_3-L_4 larvae of *An. arabiensis* (*U*=6338; p<0.001), (*U*=9043; p<0.001 for *Culex* and *U*=2834.5 (p<0.001) for pupae between the treated and untreated ponds, and *U*=988 (p<0.05) and *U*=978 (p<0.05) for *An. arabiensis* (L_1-L_2 and L_3-L_4) between the pre- and post-treatment results, but no significant difference for *Culex* and pupae).

Agnique® MMF efficacy (at a dosage of 0.25 ml/m^2) against larvae of *An. arabiensis, Culex* and pupae in the field are presented in Table 1. Examination of these data show that Agnique® MMF killed pupae faster, usually within 24 hours of treatment. L_3-L_4 instar larvae of *An. arabiensis* exhibited sensitivity similar to pupae. This fact is more evident by the fact that before MMF application (Day0) a mean density of 3.1 (L_3-L_4) *An. arabiensis*, and 0.5 pupae in the control ponds and 5.2 (L_3-L_4) *An. arabiensis*, 0.8 *Culex* and 0.7 pupae in the treated ponds.

In the first week (Day1 to Day7) after MMF application, these densities started to decline gradually for L_3-L_4 larvae from 3.1 (Day0) to 1.2 (Day1), 0.8 (Day2), 0.9 (Day3) and 0.5 on Day7 post-treatment, while the pupae in the ponds with Agnique® surface film, completely disappeared within 24 hours post-treatment. During the same time in the control ponds, *An.*

Table 1

Mean±SE of immature stages of mosquitoes collected from ponds treated with Agnique® MMF (0.25ml/m^2) and untreated controls at Bahary, Khartoum, Sudan during the 9 week testing period (3 November 2006-11 January 2007).

Time post-treatment (Days)	Control ponds				Treatment ponds			
	An. arabiensis		*Culex* spp	Pupae[a]	*An. arabiensis*		*Culex* spp	Pupae[a]
	L1-L2	L3-L4	L1-L4		L1-L2	L3-L4	L1-L4	
Pre	3.9±0.771	3.1±0.865	3.5±2.110	0.5±0.240	3.9±0.689	5.2±2.794	0.8±0.593	0.7±.351
1	3.7±0.564	3.2±0.668	3.0±0.904	1.5±0.413	2.3±0.526	1.2±0.399	0.8±0.372	0.0±.00
2	3.8±0.629	2.5±0.396	3.1±.887	1.0±0.235	1.7±0.383	0.8±0.293	0.5±0.230	0.04±.04
3	4.2±0.783	3.5±0.866	3.6±1.246	1.1±0.335	1.4±0.325	0.9±0.434	0.9±0.627	0.2±.213
7	2.7±0.480	3.1±0.588	2.6±1.074	1.0±0.339	1.6±0.507	0.5±0.209	0.3±0.127	0.2±.143
10	2.6±0.475	1.9±0.365	2.9±1.324	0.9±0.173	3.2±0.941	0.9±0.332	0.3±0.139	0.1±.106
12[b]	3.2±0.591	2.1±0.371	1.8±1.105	0.9±0.271	4.8±1.522	1.1±0.402	0.7±0.347	0.3±.200
14	3.2±0.698	1.4±0.238	2.8±1.295	0.7±0.191	2.5±0.778	0.7±0.290	0.9±0.691	0.0±.00
16	3.6±0.846	2.0±0.332	3.0±1.621	0.6±0.140	2.2±0.615	0.6±0.220	0.5±0.344	0.0±.00
18	3.8±0.631	1.7±0.354	2.3±1.337	0.5±0.112	1.6±0.406	0.6±0.292	0.3±0.218	0.0±.00
20	3.1±0.719	1.8±0.414	1.7±0.769	0.4±0.071	1.5±0.566	0.6±0.354	0.5±0.168	0.1±.047
22	3.3±0.758	1.9±0.530	2.4±0.980	0.5±0.104	1.7±0.559	0.7±0.281	0.2±0.194	0.04±.023

L_1-L_2: indicates first and second instars; L_3-L_4: indicates third and fourth instars

[a]Pupae of mixed population; [b]Second treatment round.

arabiensis L_3-L_4 instar larvae increased to 3.2 larvae/dip (Day1), 2.5 (Day2), 3.5 (Day3), and 3.1 (Day7) and the pupae ranged between 1.0 and 1.5 pupae/dip for the same sampling period (Table 1). These results indicate effective control of pupae can be obtained within 24 hours and for 3-7 days for *An. arabiensis* L_3-L_4 larvae.

The poorest results in the present study were achieved on *An. arabiensis* (L_1-L_2) and *Culex* spp larvae. Larval sampling made after 24-72 hours and at 7 days after MMF application showed densities of 2.3, 1.7, 1.4 and 1.6 larvae/dip for *An. arabiensis* (L_1-L_2) and 0.8, 0.5, 0.9 and 0.3 larvae/dip for *Culex* spp. Larval densities in the corresponding control ponds showed an increase during their period (Table 1).

In general, after the first treatment, the larval density remained low until Day7 when it started to increase reaching (*eg*, *An. arabiensis*) a peak of 4.8 (L_1-L_2), and 1.1 (L_3-L_4) on Day12. However, with reapplication of Agnique® MMF on day 13, the larval density declined again until the end of the study.

The results of the Mann-Whitney *U* test indicate no significant difference in *An. arabiensis* and *Culex* larval or pupal densities and time (*ie*, at Days 1,2,3,7 and 10 etc).

Larval and pupal percent reductions are shown in Fig 2. Pupae were completely controlled (100%) within 24 hours, then declined to 86.4% on Day7 post-treatment. With regards to the other mosquito larval stages, the percent reduction increased gradually depending on the larval development stage. The percent reduction after the pupal stages was obtained in L_3-L_4 instars of *An. arabiensis* with a reduction of 89.4% at 24 hours, 79.8% at 48 hours and 88.8% at 72 hours. The best control (92.6%) occurred at 7 days.

Lower levels of control in this study were achieved against *An. arabiensis* (L_1-L_2) and *Culex* spp larvae (all stages). Against L_1-L_2, MMF application resulted in 47.6%, 57.5% and 62.2% reductions at 24, 48 and 72 hours, respectively; against *Culex* spp larvae a maximum of 65.5% reduction was achieved 72 hours after MMF treatment. By Day7 this dropped to 57.4% (Fig 2). Similar results were obtained with the second treatment.

The results of the Mann-Whitney *U* test indicate no significant difference in larval and pupal percent reductions at 24, 48, 72 hours, and Days7, 14 and 22.

DISCUSSION

Although published literature on the field efficacy of Agnique® MMF was not available for comparative purposes, numerous field studies were conducted from 1977 to 1994, to evaluate Arosurf® MSF (identical to Agnique® MMF) as a mosquito larvicide and pupicide against different mosquito species (White and Garrett, 1977; Levy *et al,* 1982a; Mulla *et al*, 1983; Takahashi *et al,* 1984; Karanja *et al,* 1994; Batra *et al,* 2006). These studies documented that Arosurf® MSF gives satisfactory control of 4th instar anopheline species within 24 to 48 hours post-treatment and in culicine species within 48 to 96 hours. They also reported pupae were the most sensitive stage and were usually eliminated within 24 hours after Arosurf® MSF treatment.

In this field trial, Agnique® MMF at a dosage of 0.25 ml/m^2 (the lowest recommended dose), gave effective control (88.8%) at 72 hours on *An. arabiensis* (L_3-L_4) larvae and (92.6%) after 1 week, while against pupae it resulted in 100% control within 24 hours after treatment. These results are consistent with a study by Batra *et al* (2006), who tested Agnique® at a dosage of 2.0 ml/m^2 against *An. stephensi* and *An. subpictus* in water storage tanks and reported a 75% reduction (L_3-L_4) after 4 days and 100% control of pupae in 24 hours. White and Garrett (1977) reported greater then 90% control of 4th instar

larvae and pupae of *An. qaudrimaculatus* could be achieved within 24 hours after treatment with Arosurf® MSF at a dosage of 0.043 gal/acre.

The present study showed that early instar larvae (L_1-L_2) of *An. arabiensis* and *Culex* (all stages) in the field were reduced by 47.7% to 62.6% (L_1-L_2) and ranged between 63.6% and 65.6% against *Culex* spp at 24 to 72 hours, giving the lowest control level reported during the course of this study. However, the persistence of mosquitoes in early immature stages to MMF film is not surprising, because it has been shown that with Arosurf® MSF, the rate of larval mortality is related to species, instar, development within instar, habitat oxygen levels, habitat surface characteristics, habitat water temperature, and wind speed and direction (White and Garrett, 1977).

Reiter (1978) reported that in the winter when water is cool, early instars of mosquito larvae can withstand submersions for prolonged periods, making use of oxygen dissolved through the cuticle. In such situations the larvae have decreased metabolic rates (larval activity) and larval development. Le Sueur and Sharp (1988) demonstrated that low water temperatures increase dissolved oxygen concentrations (Levy *et al*, 1984). Less frequent larval surface film contact and increased cuticular respiration enhance larval survival in low water temperature treated with Arosurf® MSF. These observations may explain the lower early instar larval control reported in this trial, since it was conducted during the winter and the water temperature was low.

Dosage was also found to be important in the persistence of non-toxic physicochemical effects of MMF on larvae; the higher the dose the longer the persistence on the treated surface (Batra *et al*, 2006). In the present study, MMF was tested at the lowest recommended dosage (according to the manufacturer), in addition to the frequency disruption of MMF film by wind, waves and floating objects (*eg*, leaves, plastic) which may have contributed to the short persistence of MMF film and thus delayed larvicidal activity (Levy *et al*,1984).

This study was conducted in the winter. Twelve days application of Agnique® MMF (0.25 ml/m^2) completely controlled (100%) pupae within 24 hours and 92.6% at L_3-L_4 larvae of *An. arabiensis*. In general, the sensitivities of immature stages of mosquitoes were in the following order: pupae > instars L_3-L_4 > instars L_1-L_2. Agnique® MMF effected populations of anopheline species faster than culicine spp. However, to obtain permanent mosquito control in these breeding habitats, treatments need to be carried out at least every 7 days.

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