Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com

Original article https://doi.org/10.12980/apjtd.7.2017D7-69

Semi-field and field studies on the efficacy of monomolecular surface film (Agnique®) against immature mosquitoes in the malarious areas of Iran

Habibollah Turki[1], Aboozar Soltani[2*]

[1]*Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*

[2]*Research Center for Health Sciences, Institute of Health, Department of Medical Entomology and Vector Control, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran*

ARTICLE INFO

Article history:
Received 10 Apr 2017
Received in revised form 12 Jun 2017
Accepted 23 Jul 2017
Available online 4 Aug 20174

Keywords:
Monomolecular film
Larvicide and pupicide
Mosquito
Malaria vectors
Iran

ABSTRACT

Objective: To investigate the efficacy of monomolecular surface film (Agnique® MMF) as a new method against immature stages of *Anopheles* mosquitoes in an malaria-endemic area.
Methods: Semi-field and field trials were designed to evaluate the efficacy of Agnique® MMF as a mosquito control method in Hormozgan Province, Iran.
Results: In the semi-field trial, larval density was significantly reduced (91.80%) within 72 h post treatment with 0.47 mL/m^2 of MMF. The results showed 100% inhibition of adult emergence at 0.47 mL/m^2 for up to three weeks. Indeed, pupae were completely eliminated from all artificial ponds within one week post-treatment. In the field trial, *An. stephensi* with the frequency of 44% was the dominant species. Other collected species were *An. d'thali* (18%), *An. turkhudi* (12%), *An. moghulensis* (10%), *An. superpictus* (8%), *An. culicifacies* (5%), and *An. fluviatilis* (3%). In natural breeding places, 68.93% and 77% reductions were observed in larval density within 72 h post treatment with 0.47 and 0.94 mL/m^2 dosages, respectively.
Conclusions: In conclusion, MMF with the dosage of 0.47 mL/m^2 could be practically used as an effective larvicide against anopheline mosquitoes in malarious areas of Iran and other countries in the Middle East. MMF not only is an efficient mosquito and midge larvicide and pupicide, but also can be used as a main component of an Integrated Vector Management (IVM) strategy for controlling the vectors of mosquito-borne diseases.

1. Introduction

Mosquitoes transmitting the causative agents of several infectious diseases are considered to be one of the most important health issues. Despite extensive efforts to control mosquitoes, the prevalence rates of these diseases are still on the rise in different tropical and subtropical parts around the world[1]. Therefore, it is necessary to control mosquitoes in these areas in order to reduce the incidence of mosquito-borne diseases[1-3].

In some situations, mosquito prevention and control are the most beneficial to people in the shortest time. On the other hand, larval control is preferred in places where habitats are limited and identifiable. Therefore, combating larvae can be particularly useful in these areas[4,5].

Up to now, various methods have been used against the immature stages of vector mosquitoes. These methods include use of chemical insecticides, insect growth regulators (IGRs), larvivorous fishes, fungi, viruses, bacteria, and physical pesticides[2]. However, due to problems such as resistance because of indiscriminate use of insecticides, not being cost-effective, and some environmental problems, vector control experts decided to use non-chemical methods for mosquito control. Monomolecular surface film (MMF) is one of these non-chemical and eco-friendly methods used to control immature stages of mosquito[6,7]. By forming a uniform thin layer on the water surface, this biodegradable oil suffocates mosquito larvae and pupae, hardens adult's emergence, and prevents laying eggs in applied breeding places. Efficacy of these methods has been studied in several species of mosquitoes in different parts

*Corresponding author: Aboozar Soltani, Research Center for Health Sciences, Department of Medical Entomology and Vector Control, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran.
Tel: +989126059289
E-mails: abu2sol@yahoo.com, soltani_ab@sums.ac.ir
The journal implements double-blind peer review practiced by specially invited international editorial board members

throughout the world[8-24].

In some southern parts of Iran, most villagers use stored water for drinking and washing due to lack of the water piping system. The stored water provides ideal conditions for mosquito habitats[1]. Various re-emerged malaria epidemics have occurred in different cities of Iran in the recent years[25,26]. Iran is also at risk of some diseases caused by arboviruses, such as dengue virus, west Nile virus, and zika virus[27,28]. To date, many methods are being used for mosquito control in Iran. Depending on the situation, source reduction, biocontrol (*Bacillus thuringiensis* and larvivorous fishes), larviciding, or adulticiding may be used to manage mosquito populations[1,29].

The present comprehensive semi-field and field studies were designed for the first time in Iran in order to investigate the efficacy of monomolecular surface film (Agnique®) as a new method against immature stages of *Anopheles* mosquitoes in a malaria-endemic area.

2. Materials and methods

2.1. Study area

This study was done under semi-field and field conditions in one of the most important malarious areas of Iran, *i.e.*, Hormozgan Province. The semi-field study was conducted in Bandar Abbas Port, while the field study was carried out in Hormoodar Village in Hormozgan Province. The exact location of the study area has been shown in Figure 1.

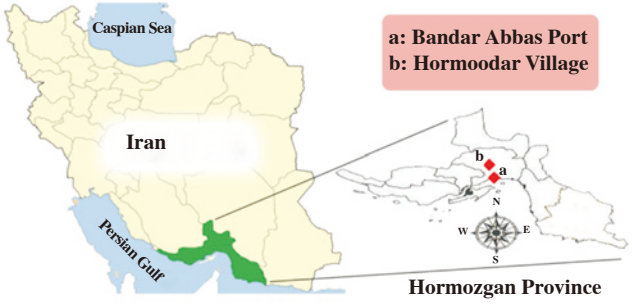

Figure 1. Map of the study areas in an malaria-endemic area, Hormozgan province, Iran.

2.2. Mosquito strains

A susceptible laboratory strain of *Anopheles stephensi* (from insectarium of Department of Medical Entomology and Vector Control, School of Health, Hormozgan University of Medical Sciences) was used in the semi-field study. In another phase of the study, all mosquitoes were wild strains. This phase of study was completely conducted under filed conditions.

2.3. Insecticide

Mosquito larvicide used in the present study was Agnique® MMF (EPA Reg. No. 7969-333), which was obtained from BASF Corporation, Research Triangle Park, NC 27709, USA. Active ingredients of this insecticide are poly(oxy-1,2-ethanediyl), α-(C16-20 branched and linear alkyl)-ω-hydroxy.

2.4. Semi-field trial

This study was carried out in 1 m × 1 m × 0.5 m artificial ponds at Bandar Abbas Port. Six concrete ponds were prepared before starting the tests. Then, artificial ponds were filled by well water in order to prepare breeding places for mosquitoes under natural conditions. Water levels in the artificial ponds were monitored and maintained at 0.4 m in depth by carefully adding freshwater during the trials (Figure 2A).

Figure 2. Concrete ponds (artificial breeding places), Bandar Abbas Port, Southern Iran (A), installed emergence traps on artificial ponds with sleeves for aspirating probable emerged adults (B) and natural breeding places of anopheline mosquitoes near Hormoodar River, Hormozgan Province, Iran (C).

Totally, five ponds were selected for adult emergence and larval density tests. All ponds were covered with emergence traps (1 m^3 of the net with 156 holes per square inch) to record the daily number of adults emerged from the respective artificial ponds and to prevent the wild population from laying eggs into the ponds (Figure 2B). It should be noted that one pond was left untreated as the control. Before adding MMF to the water surface, 100–120 larvae (I + II instars) were added to each pond. The number of larvae and adult emerged (using mouth aspirator) was recorded daily for three days. On the 3rd day, MMF was added to the ponds.

At first, a thin layer of MMF was tested for mosquito larvae and pupae of *Anopheles stephensi* under semi-field conditions. Based on the catalogue of the product, 0.47 mL/m^2 (0.5 gal/acre) of MMF was added to each pond by using a dropper. In order to reach a uniform coverage in these artificial breeding places, MMF was added to different parts of the ponds. Number of alive larvae and pupae was counted and recorded daily for two weeks. Temperature and relative humidity were also recorded every day.

2.5. Field trials under natural conditions

Before any treatments, mosquito species were collected from all breeding places near the Hormoodar River. The collected samples were transferred to the laboratory for identification based on their morphological characters by valid taxonomic keys[30].

Field evaluation of Agnique® MMF was carried out in a village near Bandar Abbas Port called Hormoodar where there is endemic malaria in south of Iran, Hormozgan Province (27°11' N, 56°16' E) (Figure 2C).

Before application, information regarding the mosquito species in the breeding sites was obtained by collecting samples of immature stages and identifying adults after emergence. Information about general topography of the area, weather conditions, water temperature, and pH of water was recorded as well. Breeding sites were selected for the field trial based on high larval populations and continued oviposition. Among the various types of mosquito breeding habitats that were available from the study area, eight breeding sites were selected for the study.

MMF was added to six selected breeding places with the highest mosquito density near the river of Hormoodar Village. The effect of MMF on mosquito density was evaluated and compared with the controls (two untreated breeding places). These trials were carried out from September to November 2015.

In this phase of the study, two doses, *i.e.*, 0.47 and 0.94 mL/m^2 (0.5 and 1.0 gal/acre) of Agnique® MMF were applied. The density of mosquito larvae before treatment was calculated in all sites for three days. After three days, MMF was added to the water surface of the selected breeding places. Density of larvae and pupae was calculated daily for two weeks after the treatment.

Larval density was measured using a standard dipper consisting of a white aluminum bowl with 9-cm diameter and 300-mL capacity[25]. A fixed number of 10 dippers were taken from the periphery and the center of the ponds. Samples of late-stage larvae and pupae were collected and brought to the laboratory for species identification after mounting.

Pre-treatment and post-treatment immature densities (I + II instars, III + IV instars, and pupae) per 10 dippers were recorded separately for both treatment and control sites. Then, efficacy and durability of this degradable oil were determined by comparing the pos-treatment counts of larvae and pupae in the treated and control sites with the pre-treatment populations.

2.6. Statistical analysis

Percent reduction was calculated using the formula described by Jiang and Mulla[31]

% Reduction = 100 – (C1 × T2/T1 × C2) × 100

where, C1 and T1 are respectively pre-treatment immature densities in control and treated sites, and C2 and T2 are post-treatment immature densities in control and treated sites, respectively[9].

Means were compared using *t*-test and ANOVA. All statistical analyses were performed using the SPSS statistical software, version 19 and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Semi-field trials

Trend of changes in number of mosquitoes in immature stages in treated and untreated ponds had been presented in Figure 3. No significant difference was found within the number of mosquitoes (larval density) in the control ponds during post treatment phase ($P = 0.426$). However, a significant difference was observed in mosquito density in the treated ponds during post treatment ($P = 0.009$).

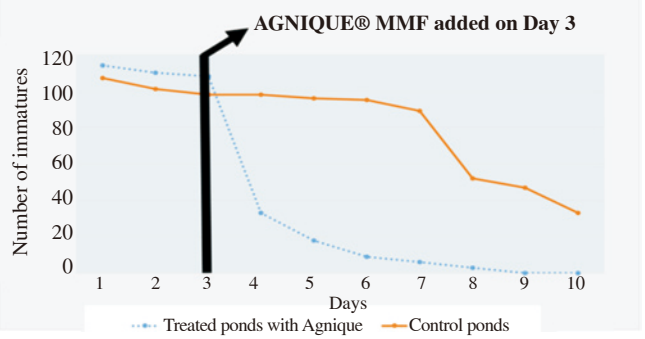

Figure 3. Trend of the number of *Anopheles stephensi* in immature stages in treated and untreated artificial ponds, Bandar Abbas, 2015.

In the MMF-treated ponds, larval density was significantly reduced (91.80%) 72 h post treatment with 0.47 mL/m^2 of MMF. The results also showed 100% inhibition of adult emergence at 0.47 mL/m^2 for up to three weeks. Additionally, pupae were completely eliminated (100%) from all artificial ponds within one week after the treatment.

3.2. Field trials

The results showed that from all collected species, *Anopheles stephensi* with the frequency of 44% was the dominant species of anopheline mosquitoes in this malaria-endemic area of Iran. Other collected species were *An. d'thali* (18%), *Anopheles turkhudi* (12%), *Anopheles moghulensis* (10%), *Anopheles superpictus* (8%), *Anopheles culicifacies* (5%), and *Anopheles fluviatilis* (3%).

Pre-treatment and post-treatment trends of larval density in treated and control breeding places have been depicted in Figure 4. Accordingly, no significant differences were found in the density of mosquitoes in control breeding sites after treatment ($P = 0.735$). However, a significant difference was observed in the density of mosquitoes in treated breeding places post treatment with two concentrations (0.47 and 0.94 mL/m^2) of MMF ($P < 0.001$) (Figure 4). Nonetheless, there was no significant difference between the results of the two applied concentrations ($P = 0.18$).

In the breeding places treated with 0.94 mL/m^2 of MMF, more than 77% reduction was observed in larval density 72 h post

treatment. At the 0.47 mL/m^2 concentration, a 68.93% reduction was observed in the same period.

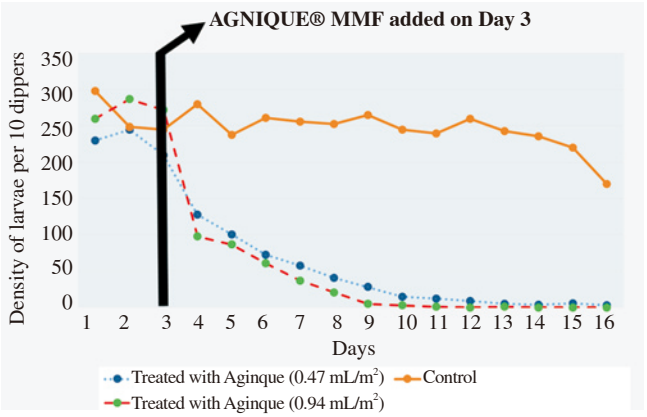

Figure 4. The larval population during 2 weeks in the eight selected breeding places (6 treated and 2 controls) near the Hormoodar River, Hormozgan Province, Southern Iran, 2015.

4. Discussion

Malaria is a major public health issue all around the world. In Iran, this mosquito-borne disease is one of the health priorities[32]. Larviciding is one of the most effective methods of vector control, especially in areas that have restricted breeding places for mosquitoes, such as southern parts of Iran[1].

The idea of using monomolecular surface film as an effective larval control method was introduced due to its usability in drinking water, relatively high persistency, and non-toxic property for non-target organisms[18]. Because of the physical mechanism, resistance to insecticides is not considered for this case[13].

Recently, many studies have been done in order to evaluate the efficacy of MMF in different medically important species in several countries[17-24]. Efficacy of Agnique® MMF was tested against immature stages of *An. stephensi* in simulated and natural habitats in India. In that study, semi-field trials carried out in cement tanks showed 100% inhibition of adult emergence at 0.4 mL/m^2 for up to 1 week and at 1 mL/m^2 for up to 3 weeks. In addition, a small-scale field trial in tanks and wells at 1 and 2 mL/m^2 led to more than 75% reduction of late instars and 100% reduction of pupae on Day 1[15]. These results were almost similar to those of the current research.

Another study was carried out in Sudan in order to evaluate the efficacy of Agnique® MMF against larvae of *An. arabiensis* and *Culex* spp. under field conditions. The results indicated that at the dosage of 0.25 mL/m^2, 79.8%–89.4% and 63.5% reductions were observed in all stages of *An. arabiensis* and *Culex* larvae, respectively. It was found that Agnique® could be more effective against immature stages of *An. arabiensis* compared to *Culex* spp. [33].

Efficacy of Aquatain, another monomolecular film, for controlling malaria vectors was also investigated in Rice Paddies in Western Kenya. The researchers reported that at the dose of 1 mL/m^2, there was a 93.2% reduction in emergence of anopheline adults and a 69.5% reduction in emergence of culicine adults. These results confirmed those of other researchers who noted that monomolecular film was more suitable for reduction in emergence of anopheline than culicine adults[34].

The present study for the first time in Iran evaluated the efficacy of Agnique® MMF against immature mosquitoes under semi-field and field conditions. The results showed that pupae could be completely eliminated (100%) within 1 week after treatment with 0.47 mL/m^2 of MMF. At this concentration, MMF resulted in a 91.80% reduction in all larval stages of *An. stephensi* under semi-field conditions 72 h post treatment. Under field conditions, on the other hand, a 68.93% reduction was observed in anopheline mosquitoes after 72 h treatment with the same dosage. However, no significant differences were found between the two applied dosages regarding the reduction rates of anopheline mosquitoes. Accordingly, it can be concluded that MMF with the dosage of 0.47 mL/m^2 can be practically used as an effective larvicide against anopheline mosquitoes in malarious areas of Iran and other countries in the Middle East.

In line with the previous studies in this field, our findings showed that Agnique® MMF had a fast and effective impact on reduction of immature stages of all anopheline mosquitoes under field conditions in malaria-endemic areas of Iran. The results also indicated that this product could replace other routine chemical larvicides that have harmful effects on environment and human health.

In conclusion, Agnique® MMF could be used in different types of mosquito habitats, including potable and irrigation waters, permanent and semi-permanent waters, irrigated croplands and pastures, and waters with outlets to natural water bodies[23]. In malarious areas of Iran, which are limited to southern parts of the country, breeding places of mosquitoes are restricted because of low average annual rainfall. Thus, this eco-friendly, effective, safe, and physical larvicide could be an ideal option to be applied for malaria elimination program in Iran and other similar countries. Overall, MMF not only is an efficient mosquito and midge larvicide and pupicide, but also can be used as a main component of an Integrated Vector Management (IVM) strategy for controlling the vectors of mosquito-borne diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Hereby, the authors would like to thank Ms. A. Keivanshekouh at the Research Improvement Center of Shiraz University of Medical Sciences for improving the writing of English in the manuscript.

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