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Aquatain™, monomolecular surface film for mosquito control in unused wells breeding site

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ABSTRACT

Water collections in Qalyubiya villages are providing suitable breeding habitats for mosquitoes including canals, unused wells (sakia pits), pools, ditches, and drainages. The impact of Aquatain™, a monomolecular surface film against nature population of mosquito larvae and pupae was tested in the unused wells in Dajwa village, Qalyubiya Governorate, Egypt. Monomolecular films are used for mosquito control because of their suffocating effect on larvae and pupae. Aquatain™ was applied according to recommended dose in six unused wells. The results showed that Aquatain had a long-lasting effect on mosquito population at a dose of 1 ml/m^2 and 0.5 ml/m^2, where it caused 100% and 97.9% larval reduction after 3 days post-treatment, respectively. While, pupal reduction reached 100% after 1 and 3 days post-treatment, respectively. Larval mortality ranged from 85.7% to 41.1% at 0.5 ml/m^2 and 93.8% to 66.4% at 1 ml/m^2. Pupal mortality ranged from 98.6% to 65.6% and 100% to 78.3% at 0.5 ml/m^2 and 1 ml/m^2, respectively. Among the larval instar, 4th larval instar was more susceptible to Aquatain (93.9%), while 1st larval instar was less susceptible to Aquatain (29%) at both doses. We noted the Aquatain™ was more effective at 1 ml/m^2 than 0.5 ml/m^2 in mosquito reduction with stability for 15 days post-treatment. The results indicated that Aquatain provides a sufficient larval and pupal control in wide mosquito breeding habitats.

INTRODUCTION

Aquatain™, monomolecular surface films is considered a new generation product of monomolecular layers. It differs from petroleum products due to their entirely physical and non-toxic mode of action (Djouaka *et al.,* 2007). It has the ability to self-spread over large water surfaces and around vegetation providing complete coverage film even in emerging aquatic plants (Batra *et al.,* 2006; Ultimate Agri-Products, 2008).

Monomolecular surface films are biodegradable, low toxicity to human and have been shown to have no adverse effects on mammals and several species of vertebrate and invertebrate aquatic organisms. Therefore, these materials are not expected to insult the environment or pose a health hazard to man (Nayar and Ali, 2003; Mbare *et al.,* 2014). Monomolecular layers differ from other mosquito control agents because of their ability to target multiple stages of mosquito life cycle and biting midges (Nayar and Ali 2003; Batra *et al.,* 2006). The monomolecular surface film acts as physical rather than chemical, because it reduces the water surface tension and thus disrupts normal development of mosquito immature. All stages of mosquitoes 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 (Nayar and Ali, 2003; Service, 2008; Sukkanon *et al.,* 2017).

Field and laboratory studies showed that products of monomolecular layers, Aquatain (AMF), Arosurf (MSF), and Agnique (MMF) are effective in controlling larvae and pupae of *Culex, Aedes, Anopheles* mosquitoes and they also suppress the adult emergence of nuisance biting midges (Nayar and Ali, 2003). Webb and Russell (2012) investigated the potential of Aquatain in backyard habitats in Australia and revealed the success of Aquatain in keeping mosquito larval habitats free from mosquito eggs, larvae and pupae up to 5 weeks post application. Kioulos and Koliopoulos (2015) tested the impact of Aquatain™ on mosquito larvae in a rice field in Central Greece and found that larval mortality ranged from 100% to 70%, 25 days after application.

Unused wells or sakia pits were used for irrigation in past time in Egyptian villages but they are neglected at the present time. These wells are suitable sites for mosquito breeding places. Unused wells were found to be the most important mosquito breeding habitat in some areas at Qalyubiya governorate (Baz, 2013). The unused wells are usually containing materials such as aquatic plants, mud, garbage, and sometime decomposing food, so they are rich nutrient sites for mosquito breeding. The presence of immature mosquito in unused wells breeding places indicated that they are strongly attracted to adult females. Mosquito larvae *Culex pipiens*, *Culiseta longiareolata, Cx. univittatus* were collected from wells, sakia pits and other polluted breeding sites (Soliman, 1985; Abdel-Hamid *et al.,* 2011; Baz, 2013). Mosquito control in field areas especially in polluted and stagnant water environments is a major concern to mosquito control districts of Qalyubiya area, Egypt. The Qalyubiya Governorate is characterized by the diversity of water sources with their topography area, agricultural, semi-desert and desert areas. Therefore, the present work aimed to evaluate the efficacy of Aquatain™, a monomolecular surface film against culicine mosquito (*Culex pipiens*) immature in unused wells.

MATERIALS AND METHODS

Study area:

The Dajwa village is located 18 km West of Tokh City and North of Cairo, Egypt. This site is adjacent to Damietta Nile Branch and surrounded by agricultural lands and citrus trees with many of water resources. This village contains a number of unused wells, where the villagers used it in irrigation. Unused wells are considered permanent or semi-permanent breeding sites for mosquitoes with stagnant water.

Description of unused well sites:

Unused wells were used for irrigation in past time; they are circular structure installed vertically on the water surface inside the water chamber, where the unused wells are less immersed as part in water chamber (Fig. 1). The circumference of the circle has a number of vessels (Aquarius) to collect the water from the water chamber and lift it to the top of the circle in order to pour water in irrigation channels. Unused wells are permanent breeding sites and usually contain materials such as mud, leaves, algae, debris, and garbage. These sites were categorized as moderately and stagnant water. Duck-weed, algae, emerging plants, and grasses were observed inside and around the wells. There are many of unused wells were built on the edge of canals, where the water is passed through the underground tube into unused wells. So, they sometimes are more depth than the depth of canals. A few numbers of mosquito larvae, *Culiseta longiareolata, Cx. antennatus* and *Cx. univittatus* were observed in unused wells.

Insecticide:

Aquatain™, monomolecular surface film (AMF) is a unique liquid contains 78% polydimethyl-siloxane (silicone) active ingredient and it was provided by the manufacturer Aquatain Products Pty Ltd., Australia (UAPs 2008). Aquatain™ is recommended for use in standing water in domestic/suburban areas such as water tanks, ponds, swimming pool, blocked drains, septic tanks and old tires. Simply, a few drops to be squeeze on top the water (1- 2 ml/m^2 of water surface). Repeat application in 4 weeks. The manufacturer's recommended application rate for mosquito control is 0.5 and 1 ml/m^2.

Field experiments:

Aquatain™ was applied directly according to the recommended dose without sprayer machine in seven unused wells (Fig. 2). In all tested sites, 0.5 and 1 ml/m^2 doses were applied separately where the seven unused wells were divided into three groups; the first and second groups treated with 0.5 and 1 ml/m^2 respectively and the last group has one an untreated control unused well using pipette around the perimeter of unused wells. The material spread rapidly forming an invisible thin film on the water surface. The doses according to the size of water in treated site, unused wells were prepared based on recommended dose. Larval and pupal densities of each site were estimated in terms of larval or pupal/dip before and after application. Mosquito samples were taken from five dips per each breeding site using stander dipper (450 ml with long handle). Larval and pupal densities were calculated as the average number of larvae or pupae per dip. Efficacy of Aquatain™ was assessed by recording larval mortality 1, 3, 6, 9, 12, 15 and 18 days after application. The obtained results were compared to larval and pupal mortality in untreated control (unused well). The experiment was repeated three times through three months August to October 2016.

Statistical analysis:

Data were statistically analyzed using System Analysis Statistics (SAS) Program, version 6.12, 1998. The effect of Aquatain™ on mosquito population densities was studied using two-way analysis of variance (ANOVA). Data were presented as mean ± standard deviations. Probability level was significant ($p<0.05$). Larval and pupal reduction (%) calculated by using (Mulla *et al.,* 1971) formula: % reduction = 100 – {(C1 × T2)/ (C2 × T1)} × 100. Where, C1 = pre-treatment immature density in control site, C2 = post-treatment immature density in control site, T1 = pre-treatment immature density in treated site and T2 = post-treatment immature density in treated site.

Fig. 1: Structure of unused wells in the past time at Egyptian villages.

RESULTS

Aquatain™, was evaluated against mosquito immature stages in six natural unused wells at Dajwa village. The toxicity of Aquatain™ against *Culex* species mosquito larvae and pupae was presented in tables (1 and 2). The treatments with Aquatain™ resulted in a higher mortality of larvae and pupae compared to untreated control. Data given in Table 1 showed that the Aquatain™ has an effect on the mosquito population, where treatment doses 0.5 and 1 ml/m^2, larval mortality reached 97.9% and 100% three days after application, respectively. The effect of Aquatain™ was continued to 15 days post-treatment, where the mortality was 41.1% and 66.4% at 0.5 and 1 ml/m^2, respectively. The susceptibilities of mosquito larvae to doses were highly significant differences ($P \leq 0.05$).

Fig. 2: Application of Aquatain™ in unused wells (Dajwa village) for mosquito control.

Data given in the same table showed that the Aquatain™ have an effect on mosquito pupae, where at low dose (0.5 ml/m^2) mortality reached 100% three days after application. At high treatment dose (1 ml/m^2) mortality reached 100% one day after application, mortality remained at 100% six days after application and ranged from 95.3% to 78.3%, 9 and 15 days after application, respectively. Efficacy of Aquatain™ has stability in treated unused wells for 12 and 15 days post-treatment with doses 0.5 and 1 ml/m^2, respectively against mosquito larvae. While, in pupal stage, the stability of Aquatain™ reached 15 days post-treatment at for both dose. (Stability means ≥50%reduction).

Table 1: Mean number (±SD) and percentage mortality (%) of *Culex pipiens* larvae in natural unused wells over a period of 18 days after application with Aquatain™.

Days post-treatment	Dose (ml/m^2)*				Control
	0.5		1.0		
	Mean No. larvae/dip**	% Reduction***	Mean No. larvae/dip	% Reduction	Mean No. larvae/dip
1	51.4±4.3	85.7^{bB}	21.2±3.1	93.8^{aA}	341.4±11.6
3	7.0±1.6	97.9^{aA}	0.0±.0	100.0^{aA}	320.6±10.4
6	36.8±4.2	90.2^{aB}	4.4±0.5	98.8^{aA}	354.4±12.5
9	74.0±5.3	75.6^{bB}	32.8±3.4	88.7^{bA}	287.8±10.3
12	121.6±4.4	61.2^{cB}	69.2±6.2	76.8^{bcA}	297.2±12.0
15	153.2±8.2	41.1^{dB}	83.4±7.3	66.4^{cA}	246.8±8.9
18	243.6±10.4	16.4^{eB}	187.0±5.6	32.7^{dA}	276.2±12.5

* Mean No. larvae/dip before treatment at 0.5 and 1 ml/m^2 (368 and 351 larvae).
**: 5 dips (450 ml water dipper was used)
***: The percent reduction was calculated by using formula of (Mulla *et al.*, 1971)
a, b, c, d: means, within the same column have the same small letters and means within the same row have the same capital letters are not significantly different (P>0.05, LSD)

Table 2: Mean number (±SD) and percentage mortality (%) of *Culex pipiens* pupae in natural unused wells over a period of 18 days after application with Aquatain™.

Days post-treatment	Dose ml/m^2*				Control
	0.5		1.0		
	Mean No. pupae/dip**	% Reduction***	Mean No. pupae/dip	% Reduction	Mean No. pupae/dip
1	1.2±.04	98.6^{aA}	0±0	100.0^{aA}	79.0±6.2
3	0.0±0.0	100.0^{aA}	0±0	100.0^{aA}	86.4±9.5
6	3.0±0.7	97.3^{aA}	0±0	100.0^{aA}	98.8±9.9
9	9.0±2.3	90.0^{abB}	4.0±0.7	95.3^{aA}	80.0±3.8
12	16.0±3.1	83.4^{bB}	9.0±1.6	90.1^{aA}	86.0±9.8
15	28.8±4.2	65.6^{cB}	17.2±2.4	78.3^{bA}	74.8±4.2
18	43.2±6.6	28.9^{dB}	31.4±4.6	45.4^{cA}	54.2±5.9

* Mean No. pupae/dip before treatment at 0.5 and 1 ml/m^2 (93 and 88 larvae, respectively).
**: 5 dips (450 ml water dipper was used)
***: The percent reduction was calculated by using formula of (Mulla *et al.*, 1971)
a, b, c, d: means, within the same column have the same small letters and means within the same row have the same capital letters are not significantly different (P>0.05, LSD)

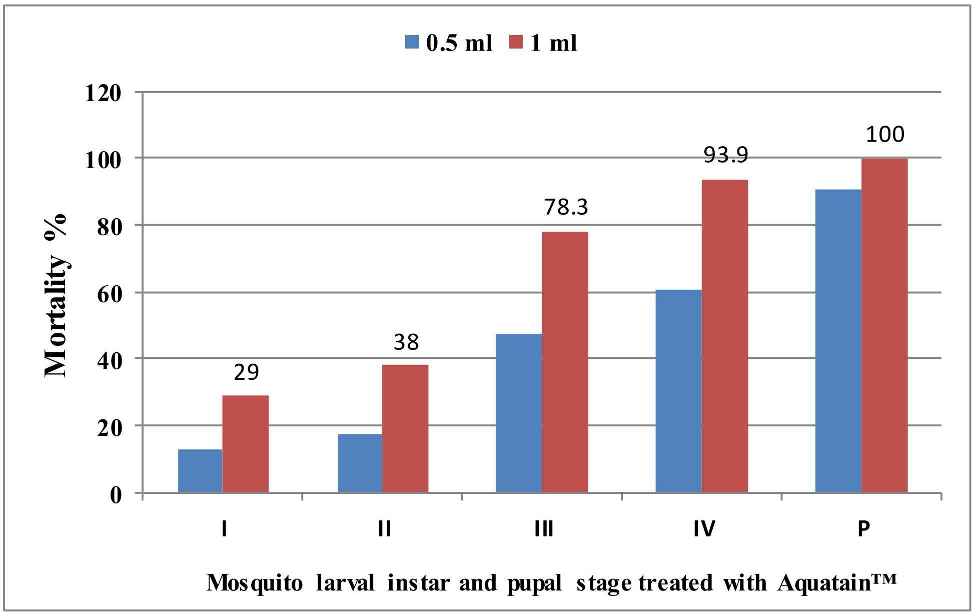

Fig. 3: The mean mortality of larval (I to IV) and pupal stages (P) treated with 0.5 and 1 ml/m^2 doses of Aquatain™ after 1 day.

Results showed that mosquito pupae were more susceptible to Aquatain™ than larval stages, where it reached 100% mortality or reduction after one and three days at 1 and 0.5 ml/m^2, respectively. Among the larval instar, 4th larval instar was more susceptible to Aquatain (93.9%), while 1st larval instar was less susceptible (29%) at both doses as mean cumulative mortalities (±SE) of the Aquatain™ treated (Fig. 3). Statistical analysis of the data using two-way analysis of variance ANOVA revealed that larval and pupal reduction at 0.5 and 1 ml/m^2 have a significant differ (P= 0.004) and (P= 0.033) respectively.

DISCUSSION

Unused wells and many water collections such as canals, irrigation channels, ditches, drainages, and pools are widely distributed in the study area. These places are considered as the most important breeding habitats for many species of mosquitoes (Baz, 2013). The unused wells contain many materials such as garbage, mud, leaves, debris, algae and decomposing food. Also, these wells are considered in some area as sewerage site, so they are rich nutrient sites base of breeding of extremely large population of *Culex pipiens*, *Culiseta longiareolata* and other species throughout the year. Aquatain™ has the ability to target multiple stages in the mosquito life cycle (Batra *et al.*, 2006). All stages that come in contact with the water surface (eggs, larvae, pupae, emerging adults and ovipositing females) are affected by the lowered surface tension caused by such layers (Nayar and Ali, 2003; Service, 2008). As a result, these layers can provide the combined benefits of larval, pupal and adult control, which leads to the reduction in mosquito density and longevity (Killeen *et al.*, 2006).

Effect of Aquatain™, on mosquitoes was evaluated in the field according to the recommended doses. Our results declared that Aquatain™, was effective and more persistence, where it caused 100% and 97.9% reduction after 3 days post-treatment at 0.5 and 1 ml/m^2, respectively with efficacy for 12 (61.2% reduction) and 15 (66.4%) days post-treatment, respectively. Our findings agree with many investigators as Webb and Russell (2009) they showed that Aquatain caused 94.6% mortality for *Cx. quinquefasciatus* and 33.6% for *Ae aegypti* larvae and 100% mortality of pupae in filed. Bukhari and Knols (2009) stated that Aquatain, compared with other films have improved spreading ability and flexibility on a water surface and at a dose of 1 ml/m^2 caused 95% mortality to mosquito immatures at one day with two weeks persistence. Bukhari *et al.* (2011) showed that Aquatain can significantly reduce larval densities and adult emergence of both anopheline and culicine mosquitoes in rice paddies without affecting other aquatic non-target organisms.

Baz (2013) who revealed that Aquatain™ had long-lasting effect on mosquito population at a dose of 2 ml/m^2 than 1 ml/m^2, where it caused 100% reduction after 2 and 4 days post-treatment in winter with stability 19-13 days, respectively, while in summer it reached 92% and 84.2% reduction for 3 days post-treatment with stability 9-7 days, respectively. Aquatain™ remained highly effective in two doses for 15 days, where the larval mortality rates ranged up to 95% and 83%, respectively (Kioulos and Koliopoulos, 2015). The present studies indicated that Aquatain™ was able to control multiple life cycle stages of *Culex pipiens* in the field. In the larval and pupal treatment, the pupal stage was more susceptible to Aquatain™ than the all larval instars. Our findings agree with many of authors (Levy *et al.,* 1982; Das *et al.,* 1986; Bashir *et al.,* 2008; Bukhari and Knols 2009; Ngrenngarmlerta *et al.,* 2016). The mortality rate was higher in late stages than young stages, because the late stages are failure to obtain the air-oxygen from water surface. Also, the surface tension was reduced after treated with monomolecular surface film (Das *et al.,* 1986; Corbet, 2000; Nayar and Ali, 2003; Senthil, 2007).

Monomolecular layer on the water may be closing off their respiratory structures; siphons in larvae and trumpets in pupae (Reiter, 1978). The increase of larval and pupal mortalities over time in two doses can be attributed to the time needed for the spread of the product and their stabilities. For this reason the effect of Aquatain™, was evident on 4th larval instar and pupal stage showed more mortality, furthermore pupae are spent more time on the water surface for breathing than larvae (Clements, 1992) and therefore they are more frequently in contact with Aquatain™ which kills them due to flooding in their respiratory tube and causing anoxia (Nayar and Ali, 2003).

Many investigators have assessed the efficacy of Aquatain, Arosurf and Agnique against mosquitoes as Levy *et al.,* (1982); Powell and Jutsum (1993); Nayar and Ali (2003); Batra *et al.,* (2006); Webb and Russell (2011); Baz (2013); Kioulos and Koliopoulos (2015); Sukkanon *et al.,* (2017). Also, many of researchers recommended the use of monomolecular films for their properties and many advantages. Monomolecular films act on mosquitoes by closing off their respiratory structures (siphons in larvae, trumpets in pupae) leading to suffocation (Reiter and McMullen, 1978). It changes 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. All larvae on the surface at the time of treatment are likely to be instantly affected because of the flooding feature. As long as the amount of Aquatain™ is enough to flood the trachea of the larvae, further increase in the concentration of Aquatain™ probably has no additional effect (Bukhari and Knols, 2009).

In conclusion, it is recommended to continue in this field of testing different natural and artificial commercial oil sources in order to use them in a wide range to overcome the huge number of mosquitoes in the farmyards and poor agricultural areas which affects the health of both animals and humans.

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ARABIC SUMMERY

استخدام الفيلم أحادي الجزيء السطحى (الأكواتين) لمكافحة الاطوار غير الناضجة للبعوض في الآبار غير المستخدمة (السواقى).

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تعتبر تجمعات المياه مكانا مناسبا لتكاثر وتوالد البعوض في قرى القليوبية بما في ذلك قنوات الري، الترعة، الآبار غير المستخدمة (السواقى)، البرك، الخنادق (قنوات صغيرة) والصرف الصحي. تم اختبار تأثير الفيلم أحادي الجزيء السطحى (الأكواتين) وهو زيت نباتى قابل للتحلل (بوليديميثيل سيليكون منشطات السطح) ضد أطوار البعوض الغير ناضجة في أماكن الآبار الغير مستخدمة (السواقى) في قرية دجوى بمحافظة القليوبية. ويستخدم الأكواتين لمكافحة البعوض بسبب تأثيره الخانق على اليرقات والعذارى وتقليل التوتر السطحى للماء. تم تطبيق الأكواتين وفقا للتركيز ذو في ٦ آبار غير مستخدمة، حيث أظهرت التجارب أن الفيلم أحادي الجزيء السطحى^{2}الموصى به ٠.٥-١.٠ ملى/م
تأثير طويل المدى على طوري اليرقة والعذراء عند تركيز ١ ملى/م^{٢} و ٠.٥ ملى/م^{٢}، حيث بلغت نسبة الاختزال فى عدد
في حين يرقات البعوض ١٠٠٪ و ٩٧.٩٪ بعد ٣ أيام من المعاملة مع إستمرارية فى الثبات ١٥-١٢ يوم على التوالي.
بلغت نسبة الاختزال فى عدد العذارى ١٠٠٪ بعد ١ و ٣ أيام من المعاملة مع إستمرارية فى الثبات لمدة ١٥ يوم على التوالي. تراوحت نسبة اماتة اليرقات من ٨٥.٧٪ إلى ٤١.١٪ عند ٠.٥ ملى/م^{٢} و ٩٣.٨٪ إلى ٦٦.٤٪ عند ١ ملى/ م^{٢}،
كما أظهرت .العذارى ٩٨.٦٪ إلى ٦٥.٦٪ عند ٠.٥ ملى/م^{٢} و ١٠٠٪ إلى ٧٨.٣٪ عند ١ ملى/م^{٢} بينما بلغت نسبة اماتة
النتائج أن الطوار اليرقى الرابع كان الأكثر حساسية لأكواتين (٩٣.٩٪)، في حين الطور اليرقى الاول كان الأقل حساسية
(٢٩٪) في كلتا الجرعات. تبين من نتائج الدراسة أن تركيز ١ ملى/م^{٢} كان مناسبا وأكثر فعالية من ٠.٥ ملى/م^{٢} على
يوما بعد المعاملة، على التوالي. 15يرقات وعذارى البعوض في الآبار غير المستخدمة مع الثبات فى الفاعلية لمدة
وأشارت النتائج أن مادة الأكواتين توفر السيطرة الكاملة على اليرقات والعذارى في أماكن توالد وتكاثر البعوض المتنوعة.