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Research article

Influence of Aquatain™, a monomolecular surface film on surface tension for controlling the filarial vector *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

Surface tension is a phenomenon in the liquid media and plays an important part in the development and survival of aquatic animals. Influence of Aquatain™ monomolecular film on surface tension was determined against mosquito larvae and pupae at different temperatures (10, 15, 20, 25, 30 and 35 °C) and Aquatain™ doses (0.5, 1.0 and 2.0 ml/m^2). In the laboratory, Aquatain™ showed larvicidal and pupicidal effects against the filarial vector *Culex pipiens*. Higher mortality was observed in late and more weighted instars/stages than young ones as well as in the pupal stage. The pupal mortality reached 76.2%, 86% and 93.3% after 12 h post-treatment at 0.5, 1.0 and 2.0 ml/m^2, respectively, and it was completely eliminated (100%) within 24 h compared to 15.1%, 26.9% and 38.2% for 1st larval instar, respectively. Also, results showed at 0.5 ml/m^2 with temperature range: 10, 15, 20, 25, 30 and 35 °C, the mortality reached 4.0, 6.7, 10.8, 17.3, 22.7, 29.3% and 32, 44, 54, 72, 84, 97.3% for 1st and 4th larval instar, respectively, where the surface tension (γ) was 65.6, 62.4, 58.0, 57.0, 54.2 and, 49.6 dyn/cm, while the Aquatain™ was more effective on mosquito larvae and pupae at high doses with the temperature range. On the other hand, without Aquatain™ dose, the mortality value ranged between 0.0 - 1.2%, and the surface tension (γ) was 74.5 dyne/cm, which is considered as an accidental death. Aquatain™ was effective against all aquatic phases of mosquitoes, especially against the last and weighted ones. Not only was the efficacy of Aquatain™ increased by increasing the dose, but it also increased with the increased temperature of the environment. This efficiency of Aquatain™ is due to its ability to reduce the surface tension of the water medium, preventing different stages of mosquitoes from reaching the surface for breathing thereby leading to suffocation and death. Therefore, we recommended Aquatain™ in programmes for mosquito control and other aquatic insects as a safe, cost-effective control agent.

1. Introduction

The monomolecular surface film consists of plant-derived oils, nonionic surfactants monomolecular film (Das et al., 1986; Nayar and Ali, 2003). Aquatain, considered a new generation product of monomolecular films, is silicone-based. It self-spreads over large water surfaces and around vegetation, providing complete coverage of a large water surface with emerging aquatic plants (Batra et al., 2006; Ultimate Agri-Products, 2008). Many researchers refer to the safety and non-toxicity of Aquatain™ making it suitable for all kinds of mosquito breeding sites (International NSF, 2008; Wang et al., 2013).

Monomolecular layers differ from other mosquito control agents because of their ability to target multiple stages in the life cycle of mosquito and other biting midges (Levy et al., 1986; Nayar and Ali, 2003; Batra et al., 2006; Wang et al., 2013), where the presence of monomolecular layer on the water surface inhibits respiration (siphons in larvae, trumpets in pupae) (Reiter, 1978) leading to suffocation (Becker et al., 2010). Many studies have shown that monomolecular films are effective against the larval, pupal and adult stage of some mosquito species (Bukhari and Knols, 2009; Webb and Russell, 2009; Ngrenngarmlert et al., 2016; Sukkanon et al., 2016; Baz, 2017; Lee et al., 2018).

Surface tension is a property of water that plays an important part in the development and survival of aquatic insects, where the surface behaves like a film as a result of its tendency to minimise its surface area. Some aquatic insects, including mosquitoes, spend some of their life stages in water and are affected by changes in the surface tension of their aquatic environment. Mosquito larvae and pupae death are caused by the presence of monomolecular films that lowers water surface tension,

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consequently leading to water entering their tracheal system and thereby causing suffocation. Pupae are significantly more sensitive to surface tension changes (Mulla and Chaudhury, 1968; Garrett and White, 1977; Koh et al., 2015). The objective of this study was to investigate the influence of Aquatain™ as a monomolecular film on surface tension in the control of mosquito larvae and pupae.

2. Materials and methods

2.1. Mosquitoes

Insectary-reared mosquito *Culex pipiens* larvae were used for all experiments in this study. *Culex pipiens* were obtained from the Medical and Molecular Entomology Section, Entomology Department, Faculty of Science, Benha University, Egypt. Mosquito larvae were reared in round enamel plates (35 × 25 × 10 cm) filled with 3 L de-chlorinated water and fed with 5–7 g wheat bran 16% protein and fish food (Tetramin®) daily. They were maintained at 27 ± 2 °C, 75 ± 5% RH under a photoperiod of 14:10 h (light/dark). Pupae were removed and adult mosquitoes were fed a 10% sucrose solution; after 3–4 days females were allowed to blood-feed on anaesthetised hamster rats with 25 female batches for 30 min. Engorged females were removed and placed in screened cages (35 × 35 × 40 cm) and provided with small cups filled with water for oviposition. Oviposited egg rafts were collected and transferred to clean enamel pans. Two developmental stages, larvae and adult females, were continuously available for the experiments and were maintained under the same laboratory conditions (Baz, 2013).

2.2. Insecticide

Aquatain™, monomolecular surface film (AMF) was provided by the manufacturer Aquatain Products Pty Ltd., Australia. Aquatain™ contains 78% polydimethylsiloxane (silicone) active ingredient and is used as larvicidal, pupicidal adulticidal agent. The manufacturer's recommended application rate for mosquito control is 0.5–1 ml/m^2.

2.3. Experimental setting

All experiments were conducted in the Medical and Molecular Entomology laboratory, Entomology Department, Faculty of Science, Benha University, at different temperatures and relative humidity. Tap water was stored in open plastic trays at least one day before every experiment to remove chlorine. Three replicates were performed according to the World Health Organization (2015) guidelines. Aquatain™ was used as recommended by the manufacturer. At the beginning and the end of the test, water surface tension, dissolved oxygen of water and temperature were measured using Cenco Surface Tension Capillary Tube (CENCO No.10) and dissolved oxygen Meter (MA 415, Yellow-Spring Instruments Company (USA).

2.4. Larvicidal effect

Aquatain™ was tested against 1st instars (L_1 age 1–2 days), 2nd (L_2 age 2–4 days) and 4th instars (L_4 age 6–8 days) separately. Twenty-five larvae were added to each of nine plastic cups (10 × 13 × 7 cm) containing 500 ml of water. Aquatain™ was applied according to recommended dose of 0.5, 1.0 and 2.0 ml/m^2, where the surface area of water was 0.027 m^2. For each instar stage, 13.5 μl, 27 μl and 54 μl Aquatain™ was added to the plastic cup (treatment), while one cup remained untreated and served as the control. Mortality was checked after 24 h. Three replicates were performed.

2.5. Pupicidal effect

Nine plastic cups (10 × 13 × 7 cm) were filled with 500 ml of de-chlorinated tap water and 25 pupae were added to each cup at the same Aquatain™ concentration as larvicidal effect section. The number of dead pupae was counted at 3, 6 and 12 h post-application. Three replicates were performed. A pupa was considered dead if it did not show the characteristic stretching reaction on slight dipping. Mortality rate was corrected by using Abbott's formula (1925).

2.6. Measurements of medium surface tension

Three different doses of Aquatain™ were tested concurrently against larvae and pupae at six different temperatures. The surface tension was measured in treated and untreated cups using a Cenco Surface Tension Capillary Tube (CENCO No.10). The capillary tubes were cleaned, dried and then placed in tested cups (500 ml) with a narrow-mouthed bottle and the height of the water as it rose within the tubes was determined.

The surface tension (γ) of water was calculated by the following equation:

$$\boldsymbol{\gamma} = \frac{\boldsymbol{\rho g h r}}{2} \tag{1}$$

where $\boldsymbol{\gamma}$ is the surface tension measured in dynes/cm, h is the height of water in cm in the capillary tube, r is the interior radius of the capillary tube in cm, ρ is the water density at the experimental temperature expressed in g/cm^3 and g is the acceleration gravity expressed in cm/sec^2.

The surface tension decrement Δγ at any temperature was calculated as

$$\Delta\gamma = \gamma_2 - \gamma_1. \tag{2}$$

where γ_1 and γ_2 respectively are the surface tension for normal water (control medium) and water after addition of the Aquatain™ at specific dosages and temperatures.

The mortality increment ΔM for each temperature was calculated as

$$\Delta M = M_2 - M_1. \tag{3}$$

where M_1 and M_2 respectively are the mortality for mosquito stage in undosed water at selected temperatures and the mortality for mosquitoes of the same stage after adding Aquatain™ at the same temperature.

2.7. Data analysis

Data analyses were performed using SPSS version 17 software (SPSS Inc., Chicago, IL). A one-way analysis of variance (ANOVA) was used to determine significant differences in the efficacy of Aquatain™ exposed to different temperatures. Median lethal time (LT50) values were calculated by Probit Analysis (Finney 1971). Data were presented as Mean ± SD, P-value of ≤0.05 was considered to have statistical significance.

3. Results

3.1. Larvicidal/pupicidal activity of Aquatain™

The influence of Aquatain™ against *Cx. pipiens* immature was evaluated (Tables 1, 2, and 3). Treatments with Aquatain™ resulted in a higher mortality of pupae and larval stages compared to untreated water. Higher mortality was observed in the pupal than larval stages at all Aquatain™ doses and selected temperatures. The pupal mortality (mean %) reached 76.0 %, 86 % and 98.2 %, 12 h after treatment compared to 0.2 % mortality for untreated water (control) at 0.5, 1.0 and 2.0 ml/m^2, respectively (Table 3).

The higher mortality was observed in the 4th instars at all doses of Aquatain™, where the mean mortality (%) reached 62.9%, 80.4% and 88.9% at 0.5, 1.0 and 2.0 ml/m^2, respectively after 24 h post-treatment (Table 3). The mean mortality (%) for 1st instars was 15.1, 26.9, 38.2% at 0.5, 1.0 and 2.0 ml/m^2, respectively, after 24 h post-treatment (Tables 1 and 2). At low treatment doses (0.5 ml/m^2), the mean mortality

Table 1. Effect of Aquatain™ on 1st instar *Cx. pipiens* at different temperatures.

Period (hrs)	Conc. (%)	Percent mortality (±SE)						Mean (%)*
		10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	
6	Control	0 ± 0^{eA}	0 ± 0^{eA}	0 ± 0^{fA}	0 ± 0^{hA}	0 ± 0^{iA}	0 ± 0^{gA}	0 ± 0^{f} (0.0)
	0.5	0 ± 0^{eD}	0.3 ± 0.3^{deCD}	0.7 ± 0.3^{efC}	1.3 ± 0.3^{gB}	1.7 ± 0.3^{hB}	2.7 ± 0.6^{fA}	1.1 ± 0.3^{e} (4.4)
	1.0	0.6 ± 0.3^{dE}	0.7 ± 0.3^{deE}	1.3 ± 0.3^{deD}	3.0 ± 1.0^{fC}	4.3 ± 0.7^{fB}	5.3 ± 0.8^{eA}	2.7 ± 0.5^{d} (10.2)
	2.0	1.3 ± 0.3^{cE}	1.7 ± 0.3^{cE}	2.7 ± 0.8^{cD}	4.7 ± 1.2^{deC}	6.3 ± 0.3^{cB}	8.0 ± 1.0^{cdA}	4.1 ± 0.7^{c} (16.4)
12	Control	0 ± 0^{eA}	0 ± 0^{eA}	0 ± 0^{fA}	0 ± 0^{hA}	0 ± 0^{iA}	0 ± 0^{gA}	0 ± 0^{f} (0.0)
	0.5	0.3 ± 0.3^{dD}	0.7 ± 0.3^{deD}	1.3 ± 0.3^{deC}	1.7 ± 0.3^{gC}	3.3 ± 0.3^{gB}	5.0 ± 0.0^{eA}	2.1 ± 0.4^{d} (8.2)
	1.0	1.0 ± 0.6^{cdF}	2.0 ± 0.6^{cE}	2.7 ± 0.6^{cD}	4.7 ± 0.3^{deC}	6.7 ± 0.3^{cB}	8.0 ± 0.5^{cdA}	4.2 ± 0.6^{c} (16.7)
	2.0	2.7 ± 0.3^{bF}	3.7 ± 0.3^{bE}	4.3 ± 0.7^{bD}	6.7 ± 0.7^{cC}	9.3 ± 0.8^{bB}	12.0 ± 1.5^{bA}	6.4 ± 0.9^{b} (25.8)
24	Control	0 ± 0^{eA}	0 ± 0^{eA}	0 ± 0^{fA}	0 ± 0^{hA}	0 ± 0^{iA}	0 ± 0^{gA}	0 ± 0^{f} (0.0)
	0.5	1.0 ± 0.0^{cdF}	1.67 ± 0.3^{cE}	2.7 ± 0.3^{cD}	4.3 ± 0.8^{eC}	5.7 ± 1.2^{eB}	7.3 ± 0.8^{dA}	3.8 ± 0.6^{c} (15.1)
	1.0	2.7 ± 0.6^{bF}	3.7 ± 0.3^{bE}	5.0 ± 0.6^{bD}	7.7 ± 0.3^{bC}	9.0 ± 1.0^{bB}	12.3 ± 1.9^{bA}	6.7 ± 0.9^{b} (26.9)
	2.0	4.0 ± 0.6^{aF}	6.0 ± 1.1^{aE}	7.7 ± 0.8^{aD}	10.3 ± 0.3^{aC}	13.3 ± 1.4^{aB}	16.0 ± 1.1^{aA}	9.6 ± 1.1^{a} (38.2)

* a, b, c, d: Means within the same column having the same small letters and means within the same row having the same capital letters are not significantly different ($P > 0.05$, LSD), * Mortality % No. of mosquito larvae/total number (25)*100.

Table 2. Effect of Aquatain™ on 4th instar *Cx. pipiens* at different temperatures.

Period (hrs)	Conc. (%)	Percent mortality (±SE)						Mean (%)
		10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	
6	Control	0 ± 0^{gA}	0 ± 0^{gA}	0 ± 0^{fA}	0 ± 0^{gA}	0 ± 0^{fA}	0 ± 0^{fA}	0 ± 0^{g} (0.0)
	0.5	2.6 ± 0.3^{fE}	3.6 ± 0.3^{fD}	5.0 ± 0.5^{eC}	9.4 ± 0.8^{fB}	9.6 ± 3.8^{eB}	15.6 ± 0.6^{eA}	7.6 ± 1.1^{f} (30.4)
	1.0	5.0 ± 0.5^{eF}	6.3 ± 0.3^{eE}	8.3 ± 0.3^{dD}	14.3 ± 0.6^{eC}	16.6 ± 0.8^{dB}	19.0 ± 0.5^{dA}	11.6 ± 1.3^{e} (46.4)
	2.0	8.0 ± 0.5^{dF}	9.6 ± 0.8^{dE}	12.0 ± 1.1^{cD}	18.0 ± 0.5^{dC}	20.6 ± 1.2^{cB}	22.6 ± 0.3^{bA}	15.1 ± 1.3^{d} (60.4)
12	Control	0 ± 0^{gA}	0 ± 0^{gA}	0 ± 0^{fA}	0 ± 0^{gA}	0 ± 0^{fA}	0 ± 0^{fA}	0 ± 0^{g} (0.0)
	0.5	5.6 ± 0.3^{eF}	7.0 ± 0.5^{eE}	8.6 ± 0.8^{dD}	14.6 ± 0.3^{eC}	17.3 ± 0.8^{dB}	20.3 ± 0.3^{cA}	12.2 ± 1.3^{e} (48.8)
	1.0	9.3 ± 0.3^{cF}	10.6 ± 0.3^{cE}	12.6 ± 0.8^{cD}	20.0 ± 1.0^{cC}	22.0 ± 1.5^{bB}	23.3 ± 1.6^{bA}	16.3 ± 1.4^{c} (65.2)
	2.0	13.6 ± 1.8^{bE}	14.6 ± 0.6^{bD}	17.0 ± 1.1^{bC}	23.3 ± 0.8^{bB}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	19.7 ± 1.2^{b} (78.8)
24	Control	0 ± 0^{gA}	0 ± 0^{gA}	0 ± 0^{fA}	0 ± 0^{gA}	0 ± 0^{fA}	0 ± 0^{fA}	0 ± 0^{g} (0.0)
	0.5	8.0 ± 0.58^{dF}	11.0 ± 1.0^{cE}	12.2 ± 1.5^{cD}	18.0 ± 1.1^{dC}	21.0 ± 1.1^{cB}	24.3 ± 0.6^{aA}	15.8 ± 1.4^{cd} (63)
	1.0	13.3 ± 0.3^{bE}	15.3 ± 0.3^{bD}	17.6 ± 0.8^{bC}	24.3 ± 0.3^{aB}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	20.1 ± 1.1^{b} (80.4)
	2.0	16.3 ± 0.8^{aE}	19.3 ± 1.2^{aD}	22.6 ± 1.4^{aB}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	22.2 ± 0.8^{a} (88.8)

Table 3. Effect of Aquatain™ on *Cx. pipiens* pupae at different temperatures.

Period (hrs)	Conc. (%)	Percent mortality (±SE)						Mean (%)
		10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	
3	Control	0 ± 0^{jA}	0 ± 0^{jA}	0 ± 0^{lA}	0 ± 0^{kA}	0 ± 0^{jA}	0 ± 0^{iA}	0 ± 0^{j} (0.0)
	0.5	4.0 ± 1.1^{gF}	4.6 ± 0.8^{gE}	8.3 ± 0.3^{iD}	12.6 ± 1.4^{gC}	17.0 ± 0.5^{eB}	21.0 ± 1.0^{cA}	11.2 ± 1.5^{g} (44.8)
	1.0	7.0 ± 0.5^{eE}	6.6 ± 0.6^{fE}	13.3 ± 1.6^{fD}	16.0 ± 0.5^{eC}	21.0 ± 1.0^{cB}	24.3 ± 0.6^{aA}	14.7 ± 1.6^{f} (58.8)
	2.0	10.3 ± 0.8^{cE}	10.3 ± 2.7^{dE}	17.0 ± 1.5^{eD}	20.6 ± 0.6^{cC}	24.3 ± 0.6^{aB}	25.0 ± 0.0^{aA}	17.9 ± 1.5^{d} (71.6)
6	Control	0 ± 0^{jA}	0 ± 0^{jA}	0 ± 0^{lA}	0 ± 0^{kA}	0 ± 0^{jA}	0 ± 0^{iA}	0 ± 0^{j} (0.0)
	0.5	6.6 ± 1.4^{eF}	9.6 ± 2.3^{dE}	14.0 ± 0.5^{fD}	18.3 ± 1.4^{dC}	22.3 ± 1.4^{bB}	25.0 ± 0.0^{aA}	16.0 ± 1.6^{e} (64)
	1.0	9.3 ± 1.3^{dE}	12.6 ± 0.8^{cD}	18.3 ± 0.8^{dC}	22.3 ± 1.4^{bB}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	18.7 ± 1.4^{cd} (74.8)
	2.0	14.0 ± 1.1^{bE}	16.6 ± 1.6^{bD}	21.0 ± 0.5^{cC}	24.3 ± 0.6^{aB}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	21.0 ± 1.0^{b} (84)
12	Control	0.3 ± 0.33^{A}	0 ± 0^{jA}	0.3 ± 0.3^{lA}	0 ± 0^{kA}	0.3 ± 0.3^{jA}	0.3 ± 0.3^{iA}	0.2 ± 0.1^{j} (0.8)
	0.5	10.6 ± 0.3^{cE}	13.0 ± 1.1^{cD}	19.0 ± 0.5^{dC}	21.6 ± 0.8^{bB}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	19.0 ± 1.36^{c} (76)
	1.0	14.6 ± 0.6^{bE}	16.6 ± 1.6^{bD}	22.6 ± 1.4^{bC}	25.0 ± 0.0^{aB}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	21.5 ± 1.0^{b} (86)
	2.0	20.0 ± 0.5^{aE}	20.6 ± 1.2^{aD}	24.3 ± 0.6^{aC}	25.0 ± 0.0^{aB}	25.0 ± 0.0^{aA}	25.0 ± 0.0^{aA}	23.3 ± 0.5^{a} (93.2)

was 15.1% and 62.8% for 1st and 4th instars, respectively, after 24 h exposure and 76.0% for pupal treatment after 12 h exposure, respectively. At 2.0 ml/m^2 treatment the mean mortality was 38.2% and 88.8% for 1st and 4th instars, respectively, after 24 h post exposure and 93.2% for pupal treatment after 12 h exposure, respectively (Tables 1, 2, and 3).

Aquatain™ reduced survival of immature stages of *Cx. pipiens* based on the median lethal time (Table 4). The mortality rates of pupae and 4th instars were higher over a short period compared to young instars (LI), where the median lethal times (LT_{50}) were 0.55 and 3.50 h for pupal and 4th larval stage at the highest temperature (35 °C), respectively,

Table 4. Efficiency of Aquatain™, monomolecular surface film against 1st and 4th instars and pupae of *Cx. pipiens* at different temperatures.

Mosquito stage	Temperature	LT_{50} (hr.)	LT_{90} (hr.)	Slope	P	R
1st instar	10	265.41	2975.30	1.2210 ± 0.5205	0.5574	0.9665
	15	191.79	2419.77	1.1641 ± 0.4447	0.9788	1.0000
	20	108.50	1076.11	1.2862 ± 0.4087	0.9640	0.9999
	25	77.35	767.16	1.0202 ± 0.3424	0.6447	0.9882
	30	55.19	547.38	0.9640 ± 0.3231	0.8490	0.9981
	35	25.65	247.61	1.3015 ± 0.3139	0.7193	0.9963
4th instar	10	20.61	142.28	1.5275 ± 0.3139	0.7249	0.9976
	15	15.75	102.01	1.5798 ± 0.3090	0.9840	1.0000
	20	11.30	70.13	1.6162 ± 0.3077	0.7987	0.9989
	25	5.32	15.86	2.7010 ± 0.2349	0.0612	0.9897
	30	4.53	11.60	3.1375 ± 0.5175	0.1512	0.9692
	35	3.50	9.86	2.8494 ± 0.5612	0.2414	0.9821
Pupae	10	8.91	80.62	1.3397 ± 0.3065	0.3694	0.9799
	15	6.46	35.07	1.7442 ± 0.3099	0.4703	0.9924
	20	2.79	12.03	2.0216 ± 0.3460	0.6432	0.9973
	25	2.75	5.62	3.2649 ± 0.5437	0.2253	0.9758
	30	2.12	3.32	6.5865 ± 2.5429	1.0000	0.8660
	35	0.55	1.67	2.6349 ± 1.8179	0.4912	0.8660

R tabulated = 0.997.

compared with 1st instars (LT_{50} = 25.65 h). These results indicated that pupal stage was more susceptible to Aquatain™ than larvae (13 h for killing at 35 °C) (Table 4).

3.2. Effect of temperature and Aquatain™ dose on the surface tension

The different values of surface tension (γ) measurements at selected temperatures and Aquatain™ doses are shown in Table 5. Data describe the relationship between the surface tension (γ) and the temperature (°C) of the aqueous medium for different doses of Aquatain™. The surface tension values for untreated water medium (control) ranged from 70.3 to 74.5 dyne/cm for selected temperatures. Under these conditions, the surface tension becomes strong and coherent, which allows the adult mosquito to stand on the water surface for oviposition or rest (Figure 1a) and allows larvae and pupae to remain at the water-air interface for respiration (Figure 1b,c).

The data show that surface tension and temperature are inversely proportional. At low temperature (10 °C) the surface tension values were 73, 70 and 65.6 dyne/cm at dose 0.5, 1.0 and 2.0 ml/m^2, respectively, compared to untreated water 74.5 dyne/cm, while, at 35 °C, the surface tension values were 61.4, 56 and 49.6, respectively, compared to 70.3 dyne/cm for untreated water (Table 5).

Results show that the relationship between water temperature and dissolved oxygen was inversely proportional (Table 5). The relationship between surface tension decrement $\Delta\gamma$ (Eq. (3)) and temperature at different Aquatain™ doses was directly proportional (Figure 2a), whereby, at every applied Aquatain™ dose, the temperature increased as well as the surface tension decrement $\Delta\gamma$ increased, i.e. the surface tension was inversely preoperational with temperature and Aquatain™ doses (Figure 2b). Also, at every selected temperature, the surface tension decrement $\Delta\gamma$ increased with added dose. At the same time, the surface tension decrement $\Delta\gamma$ increased with an increase in temperature and Aquatain™ dose, leading to drowning of the adults and suffocation of larvae (Figure 1d), because the surface tension became weak and unable to bear the adult mosquito or allow the larvae to remain at the water surface.

Table 5. Variation of surface tension (γ) measurements with temperature (°C) at different Aquatain™ doses (ml/m^2).

Temp. (°C)	DO_2 (ppm)	Aquatain™ doses (ml/m^2)			
		0*	0.5	1.0	2.0
10	12.8	74.5	73.0	70.0	65.6
15	11.2	73.7	70.7	66.7	62.4
20	10.5	72.8	69.0	63.9	58.0
25	9.0	72.0	66.8	62.5	57.0
30	8.1	71.2	63.7	59.3	54.2
35	6.8	70.3	61.4	56.0	49.6

* Control: untreated water medium.

3.3. Effect of temperature on mosquito mortality

The relationship between the effects of temperature and dosage of Aquatain™ on mortality is shown in Figure 3, for the untreated water (control). There was no significant effect of temperature on mortality. The mortality for different dosages of Aquatain™ increased as temperatures increased for all mosquito stages. As the dose of Aquatain™ was increased for each temperature, the mortality rate for each of the stages also increased. In addition, when comparing the mortality rate for each selected mosquito stage and doses, increasing temperature resulted to significant increases in mortality rate. This indicates that temperature increasing would enhance Aquatain™ efficacy as a surface monolayer and consequently improve mosquito mortality.

3.4. Effect of surface tension on mosquito mortality

Effect of surface tension on mortality based on the effect of Aquatain™ dose and temperature and the mortality as a function of surface tension decrement $\Delta\gamma$ for selected mosquito stages were plotted at different temperatures and Aquatain™ dose, as shown in Figure 4, where the figure represents the change in ΔM (Mosquito mortality increment, Eq. (3)) with change in $\Delta\gamma$ (surface tension decrement, Eq. (2)) side by side with Aquatain™ dose at different temperatures. The figure shows that mosquito mortality increases with increasing surface tension decrement in addition to both Aquatain™ dose and temperature. Moreover, the effect of surface tension is more pronounced (is apparent) at higher stages than at earlier (smaller) stages (Figure 4a). This behaviour of mortality (M %) values continues until it reaches complete mortality (100% mortality), particularly at higher life stages (Figure 4b,c). Also, the least value (the beginning of the M% values) at lowest selected

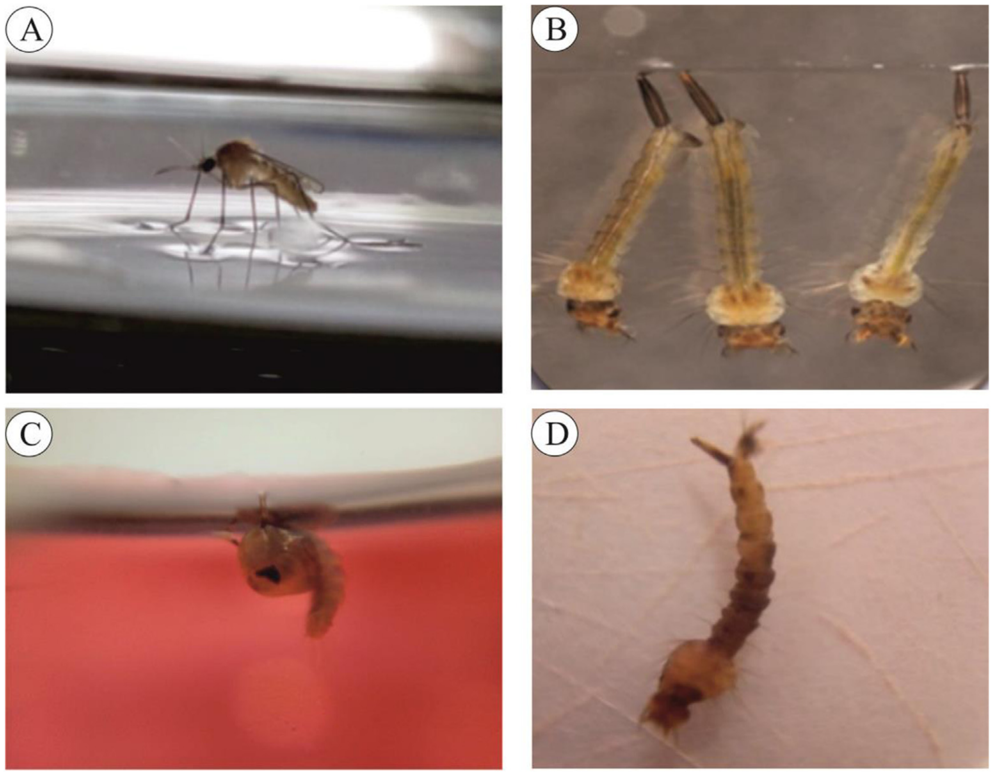

Figure 1. Untreated (A,B) and treated (C,D) *Culex pipiens* mosquito larvae and pupae with Aquatain™, (A) adult mosquito resting on untreated surface, (B) larvae breathing the oxygen through untreated surface, (C) Pupa tries to penetrate the treated surface for breathing oxygen (D) Suffocation of larvae.

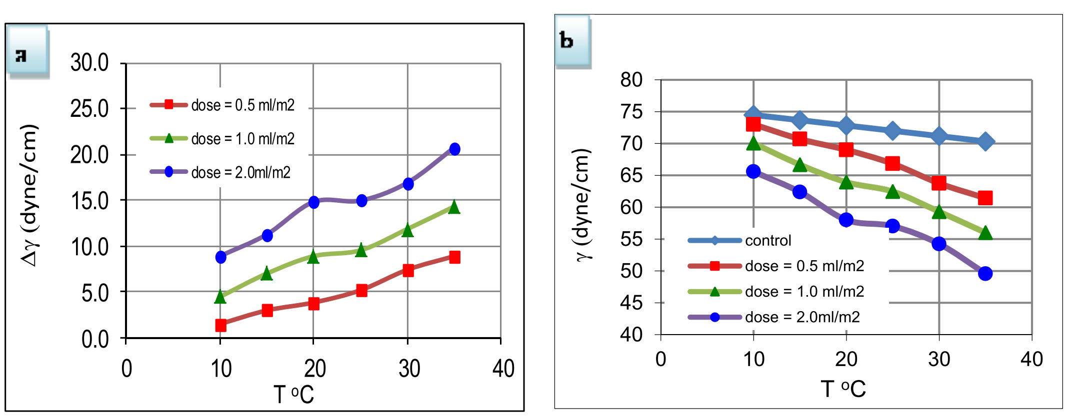

Figure 2. Variation of surface tension decrement Δγ and temperature (°C) at different Aquatain™ doses ml/m^2, (a) surface tension decrement **Δγ**, (b) surface tension (**γ**).

temperature of any stage increases with increasing life stage. Nevertheless, in complete extermination of mosquito (100% mortality), the curve of **Δγ** (surface tension decrement) and ΔM (change of mortality percentage) has a linear proportional behaviour for all life stages at all selected temperatures and Aquatain™ doses, whereas the relation is linear directly proportional.

The fitting of these proportionality parts of the curves as a linear relationship between Δγ and ΔM% was performed by using linear fitting of Microsoft Excel software (Office 365 version) giving the describing linear generalised equation (Eq. (4)).

$$\mathbf{\Delta M = s\Delta\gamma + C} \tag{4}$$

where ΔM is the increment in mortality percentage, S is the slope of the fitting line,Δγ is the surface tension decrement and C is constant. However, average value and standard deviation of both S and C constants of fitting lines for all curves in the previous figure are listed in Table 6, where Figure 4d describes the change in both S and C during mosquito age stage progress. It is observed that, as mosquito stages progress, the average slope value of the straight line equation increases. This means that the ratio (S = ΔM/**Δγ**) of increasing mortality ΔM to the decrement of surface tension Δγ increases directly with the progress of mosquito age stage. Therefore, this allows to conclude that mortality is a direct function of surface tension; it is beneficial to increase the mortality rate relative to the surface tension change rate.

4. Discussion

Monomolecular surface films have become widely used in the field of mosquito control and other medical insects for its many advantages; it is spread spontaneously and rapidly over a water surface to form a uniform

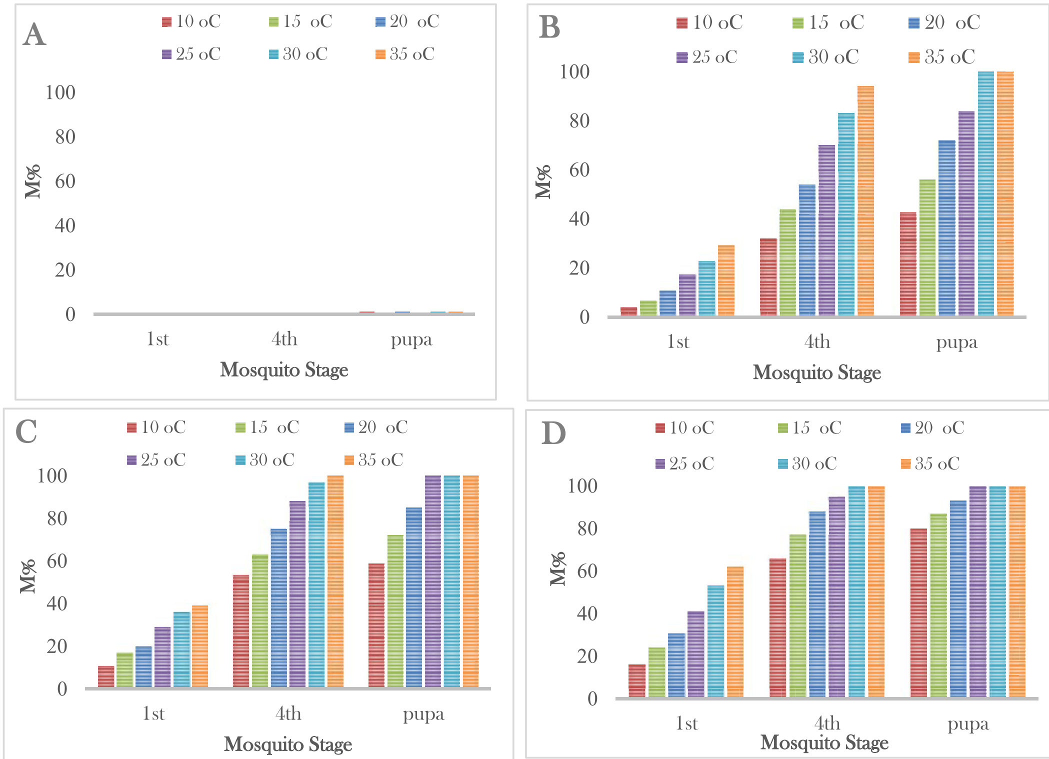

Figure 3. Mosquito mortality (M%) variation with mosquito life stages for (A) control group, (B) and at Aquatain dose of 0.5 ml/m^2, (C) 1 ml/m^2 and (D) 2 ml/m^2.

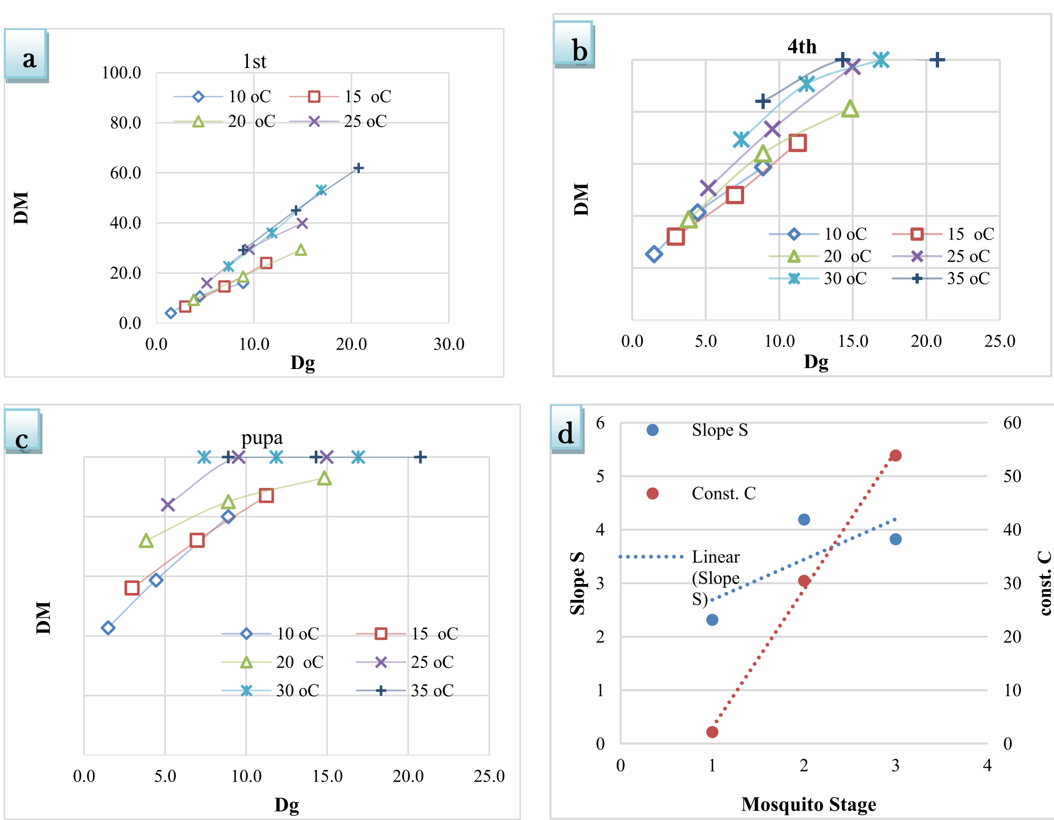

Figure 4. Mosquito mortality increment (ΔM%) variation with surface tension decrement Δγ at selected temperatures for different mosquito life stages (a)1st, (b) 4th and (c) pupa stage, whereas (d) is the average value of both S and C constants of fitting lines for all curves at different mosquito stages.

Table 6. The average value and stdv of both S and C constants of fitting lines for all curves at different mosquito stages.

	Mosquito stages							
	1st		2nd		4th		pupa	
	s	c	s	c	S	c	s	c
average	2.314617	2.184133	3.0742	2.778367	4.1904	30.4515	3.823375	53.8705
stdv	0.608401	2.457153	1.0106292	4.580775	0.69663	14.45183	1.171496	17.21754

ultrathin film with the ability to target multiple immature mosquitos and other insects that depend on the water surface for respiration (Garrett and White, 1977; Das et al., 1986; Corbet et al., 2000; Nayar and Ali, 2003; Batra et al., 2006; Ultimate Agri-Products, 2008; Mbare et al., 2014; Baz, 2017).

Monomolecular surface films and their derivatives have been developed over recent years to replace petroleum oils and serve as environmentally safe pesticides for mosquito control. Furthermore, they have been shown to be relatively safe and non-toxic to many non-target and aquatic organisms (Nayar and Ali, 2003; Batra et al., 2006; International NSF, 2008; Bukhari et al., 2011; Wang et al., 2013; Mbare et al., 2014; Ngrenngarmlert et al., 2016; Baz, 2017).

Aquatain™ demonstrated a significant role in altering surface tension that greatly reduced *Cx. pipiens* immature stages under laboratory conditions at different temperatures. Aquatain™ reduced immature survival with the pupal stage being more susceptible, followed by 4th and 1st instars. We noted that higher mortality occurred in heavy and late instars (pupal stage) than the early ones (1st) where the pupal mortality reached 93.3 % through 12 h post-treatment at high Aquatain™ doses (2.0 ml/m^2) and eliminated 100 % within 24 h compared to 38.2 % mortality for the 1st instars after 24 h at the same concentration.

These findings agree with previous results (Batra et al., 2006; Webb and Russell, 2012; Baz, 2017; Sukkanon et al., 2017). Ngrenngarmlert et al. (2016) showed that mortality of pupae reached 99.17 at 1.0 ml/m^2 in 24 h post-treatment with silicone-based monomolecular film (MMF) and it was significantly greater than that of first larvae instar (11.7 %).

The results showed that the relationship between the surface tension (γ) and the temperature (°C) of the water medium at different doses of Aquatain™ was inversely proportional, which agrees with Bukhari and Knols (2009) and Rozilawati et al. (2016).

However, no significant effect of temperature on mortality was observed in all stages of the mosquito without doses, where mortality was rare and within the normal range at water surface tension (γ) of 74.5 dyne/cm (untreated water). This explains the strength and consistency of surface tension which allows the adult mosquito to land on the water surface to rest and also helps the aquatic immature mosquito (larvae and pupae) to breathe atmospheric air through clinging to the water surface. Once the Aquatain™ surface film was applied, the surface tension dramatically decreased and recorded greater decrease as temperature increased. This indicates that increasing temperature enhances Aquatain™ efficiency of increasing mortality rate.

Levy et al. (1984) evaluated the effect of low temperature on the mosquito larvicide and pupicide Arosurf (monomolecular surface films); he declared that the rate of spreading of Arosurf film on surface depends upon the water temperature. Also, the spreading rate increased with increasing of temperatures and, therefore, increased larval mortality.

Also, we noted that, as mosquito life progresses, the mosquito mortality increased until reaching 100 % with high temperature and Aquatain™ dose, particularly for the 4th larval instar and pupal stage. While mosquito larvae and pupae grow, their body weight as well as the amount of oxygen they need to survive increases; therefore, it needs to reach the surface and cling to gain a higher amount of than young larval instars. In the presence of the Aquatain™, the mosquitoes are unable to adhere to the water surface, due to the surface tension decrement, which inhibits mosquito larvae and pupae stages to gain enough of either dissolved or atmospheric oxygen, thereby leading to drowning and suffocation. Additionally, the cuticle thicknesses of 4th larval instar and pupal stage inhibits them from absorbing the dissolved oxygen. Mosquito larvae and other aquatic insects obtain dissolved oxygen from water in addition to atmospheric oxygen (Clements, 1992; Watt, 2000) and, in the absence of the monomolecular films, mosquito larvae were not affected by reduced or scarcely dissolved oxygen (Gophen, 1985; Dale et al., 2007; Lancaster and Downes, 2013) because the atmospheric oxygen was readily accessible.

The efficiency of Aquatain™ is due to its ability to reduce the surface tension of the water medium, preventing different stages of mosquitoes from being able to cling on to the water surface to take in the oxygen (Nayar and Ali, 2003; Mbare et al., 2014; Baz 2017). Soltani et al. (2012) evaluated the effectiveness of two types of polystyrene beads, expanded polystyrene beads and waste polystyrene chips on the surfaces of pools, for mosquito control and found it resulted to mosquito density decrease (78 and 86%, respectively) after two weeks of treatment and, therefore, recommended them in mosquito control programmes. Garrett and White (1977) showed that mosquito larvae death was due to the formation of a monomolecular film which lowered water surface tension and caused larvae suffocation and also resulted in water entering their tracheal system. Further, Clements (1992) stated that Aquatain™ weakens the surface tension of the water and, therefore, the surface film floods into mosquito larvae's respiratory tube causing anoxia. Bukhari et al. (2011) indicated that Aquatain™ can be considered as a mosquito control agent in artificial aquatic habitats like rice paddies because it alters the aquatic medium physical properties.

At higher stages and higher doses, the mortality (M %) reaches 100% and, therefore, the relationship curve between mortality and surface tension decrement shows as a horizontal straight line. Far from that, the relationship curve seems to be a direct proportional straight line and, by interpreting the experimental data, we observed that, at constant temperature, the higher the Aquatain™ dose, the less the surface tension and the greater mortality will be. At constant Aquatain™ dose, the higher the temperature, the less the surface tension and the greater the mortality. Likewise, at constant Aquatain™ dose, the higher the temperature, the less the amount of dissolved oxygen in water the greater the mortality will be. This means that Aquatain™ dose, water temperature and surface tension have a direct relation on mortality of immature mosquito stages.

To sum up, since the Aquatain™-free group (zero dose) showed lower mortality in the temperatures mentioned in the research, temperature change, as a direct factor, could not, therefore, have caused the high rate of mortality in Aquatain™ dosed groups, but, in fact, was as a result of a change of surface tension due to addition of Aquatain™ doses.

5. Conclusion

The findings can be listed as follows;

- Decreased surface tension is the main property resulting in mosquito mortality.
- The highest mortality was observed in late instars (pupae and 4th larvae).
- Late and more weighted mosquito stages are suffocated due to decrement in the surface tension and the thickness of cuticle.

Finally, we are looking forward to more future studies on the effect of Aquatain™ as an alternative control against aquatic insects and other aquatic creatures.

Declarations

Author contribution statement

Abdel-Fattah D. Dawood:Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mohamed M. Baz:Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Moustafa I. Ibrahim: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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