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Susceptibility status of larval Aedes aegypti mosquitoes in the Western Region of Saudi Arabia

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Susceptibility status of larval *Aedes aegypti* mosquitoes in Saudi Arabia

Abstract

Vector control programs worldwide are facing the challenge of mosquitoes becoming resistant to available insecticides. Larviciding is a crucial preventative measure for dengue control but data on insecticide resistance of larval *Ae. aegypti* in the Middle Eastern Region are limited. This study assesses the susceptibility status of *Ae. aegypti* collected from the two most important dengue foci in Saudi Arabia, Jeddah and Makkah, to important chemical and biological larvicides; the organophosphate temephos and *Bacillus thuringiensis israelensis*, *Bti*). Whilst worldwide, and particularly in Latin America, high-level resistance to temephos is common, Jeddah and Makkah populations exhibited full susceptibility to both temephos and *Bti*. These data suggest each can be considered by vector control programs for preventative dengue control in the region, as part of temporal rotations or spatial mosaics to manage insecticide resistance.

Key words: Mosquito larvae, Larval bioassay, *Bti*, temephos

Introduction

In Saudi Arabia, insecticides are extensively used to combat mosquito-borne diseases and other household pests, as well as in agriculture (1). *Aedes aegypti* is primarily controlled by larvicides such as Spinosad (Natular®), *Bacillus thuringiensis israelensis* (*Bti*) toxin (VectoBac®), pyriproxyfen and diflubenzuron. Adulticides such as deltamethrin, permethrin, cyfluthrin and fenitrothion are also used for fogging and indoor residual spraying to reduce the density of adult mosquitoes during outbreak situations (2). Temephos, *Bti*, Spinosad and insect growth regulatory hormones such as pyriproxyfen are used as larvicides in breeding sites, but *Bti* and Spinosad are more common in Jeddah and Makkah (1, 3, 4). However, the extensive use of chemical insecticides has led to the development of insecticide resistance in *Ae. aegypti* worldwide including Saudi Arabia (4-11). In 2011, *Ae. aegypti* strains from Makkah were found to be resistant to lambda-cyhalothrin, deltamethrin, permethrin, bendiocarb and cyfluthrin (10, 11) but still susceptible to pirimiphos-methyl (actellic) and *Bacillus thuringiensis israelensis Bti* (Bacilod) (11). In addition, Jeddah strains showed high prevalence of resistance to the pyrethroid deltamethrin and permethrin and the carbamate bendiocarb (10) but no studies to date have been considered larvicides. In Jazan, the population was resistant to lambda-cyhalothrin, DDT, bendiocarb and showed moderate resistance to permethrin, deltamethrin and fenitrothion (yet remained susceptible to cyfluthrin) (4). The larvae were reported as highly resistant to temephos, but the documented LC_{50} of 61.8 mg/L, appears unfeasibly high being far beyond the LC_{50} reported for other temephos resistant populations in the world (12, 13) suggesting further investigation is essential.

A major limitation of the control program in the region is the limited surveillance to monitor the effectiveness of control intervention, or changes in the resistance of populations that may undermine the control efforts. We therefore assess the susceptibility status of the sole local dengue vector *Ae. aegypti* collected from Makkah of Saudi Arabia to larvicides (temephos and *Bti*). The outcome of this study will provide reliable, updated data on the resistance profile of larval *Ae. aegypti* populations from Saudi Arabia and may provide indication of which insecticides may be more effective.

Materials and Methods

Mosquito strain

Aedes aegypti larvae were collected from multiple breeding sites in two dengue endemic areas in Makkah (Lab= 21°45'2.13 N, 39°92'1.96 E; field=21°40'7.70 N, 39°86'3.19 E) and Jeddah (Lab=21°35'2.13 N, 39°13'9.42 E; field=21°60'3.97 N, 39°27'2.49 E). The lab strains were fifth generation from the original field which was collected in 03-04/2016. The field strain was collected in 01-02/2018. The larvae were reared as described by (10). Two reference strains, Cayman, a multiply resistant lab strain, though reported as lacking temephos resistance (14), and the standard (ubiquitously-susceptible) strain New Orleans were used. All strains were raised under the same standard insectary conditions at the Liverpool School of Tropical Medicine (10).

Larval Bioassays

Larval bioassays were carried out on *Aedes* strains shown in **Table 1** according to the WHO protocol (15) to determine the lethal concentrations (LC_{50}) and the resistance ratio relative to New Orleans (RR_{50}).

<Table 1>

Bioassays were performed using temephos (Sigma-Aldrich, Dorset, UK), or *Bti* (Vectobac®12AS 1.2%,1200 ITU/mg. A total of eight different concentrations of *Bti* and nine of temephos were used for each strain **(Table 2&3)**. The concentrations were selected as they have been reported to result in larval mortality between 10% and 95% (15). The data was used to calculate the lethal dose that kills 50% (LC_{50}) in each population. Dilutions of temephos (stock dissolved in absolute ethanol) with distilled water up to a total volume of 200mL are detailed in **Table 2.** For each concentration of each insecticide, three or four replicates of a pool of approximately 25 late third or early fourth instar larvae were tested along with a negative control pool;1mL absolute ethanol mixed into 199 mL of distilled water for temephos or into 100ml of distilled water for *Bti* assays.

<Table 2>
<Table 3>

Vectobac stock (1.2%) was diluted by adding 1ml of the stock (1.2%) to 99 ml distilled water to obtain 0.012% (120pmm) which was used in the experiment.
All larval bioassays were performed in 6 cm in diameter plastic bowls; Mortality was recorded after 24h of exposure. Any larvae failing or unable to swim up to the surface independently were counted as dead. Any larvae that had pupated during exposure were omitted from the total count.

Statistical analysis

The mortality (%) was calculated for the number of mosquitoes or larvae that were dead after 24h exposure. The LC_{50} value for the larval bioassays was calculated using probit regression analysis (SPSS version 24). If chisq >0.05, confidence limits were adjusted accordingly (SPSS does this unless the fit is terrible, if it is it will not calculated Cis). The resistance ratio (RR) was calculated by comparison of the resistant Makkah and Jeddah strains against the susceptible New Orleans strain using the formula below to monitor the level of insecticide resistance in a field population.

$$\text{Resistance ratio (RR)} = \frac{LC_{50} \text{ of resistant strain}}{LC_{50} \text{ of susceptible strain}}$$

Results

Larval bioassays

Mortality was not observed in any strain in the control assays. Based on the mortality rate across different concentrations of temephos and *Bti*, resistance to the larvicides was higher in field strains when compared to the New Orleans strain (**Table 4 and Table 5**).

<Table 4>
<Table 5>

Indeed in both the temephos bioassays, the LC_{50} confidence intervals were not overlapping in comparisons of any strain, indicating a significant difference in mortality between the strains (**Table 6)**. However, whilst there is significant variation in susceptibility, current guidelines (16), suggest that a resistance ratio <5 indicates limited/no resistance; 5-10 moderate resistance, and >10 is substantial resistance. Therefore, based on this classification, no definitive resistance to temephos and *Bti* was identified in any of the strains tested.

<Table 6>

Discussion

The current study was conducted to assess the susceptibility of larval *Ae. aegypti* to commonly used insecticides in the cities of Jeddah and Makkah. Larval bioassays did not detect resistance in either Makkah or Jeddah to temephos or *Bti* (all resistance ratios <5 compared to a standard susceptible strain (17). In contrast, extreme temephos resistance in *Ae. aegypti* larvae from Jazan (LC_{50}=61.8 mg/L) was reported in 2016 (4). When compared to the average LC_{50} of multiple separate studies of the susceptible reference strains Rockefeller, New Orleans and Bora Bora (18), this equates to a resistance ratio above 10,000, far exceeding the highest ratio of 224 previously recorded (in Brazil; (18)). This estimate from Jazan thus appears unlikely

to be correct, and in the absence of additional data, a provisional assessment of temephos susceptibility in Saudi Arabia seems appropriate.

Temephos resistance in *Ae. aegypti* larvae has been recorded globally including British Virgin Islands (19), Thailand (20), Brazil (21), Cuba (22), Colombia (23), Martinique (24) and Santiago island (25). Whilst the current data suggest susceptibility, it is important to note that both Saudi Arabian strains showed significantly higher LC_{50} values than the susceptible New Orleans strain.

Bacillus thuringiensis israelensis (*Bti*) is a bacterial derived toxin that has been widely used for vector control. The populations from Jeddah and Makkah were susceptible to this compound in comparison (based on a resistance ratio <5) with the New Orleans strain. Almost all other studies have reported similar findings, including Martinique populations (highly resistant to most insecticides) that were susceptible to *Bti* compared to the Bora-Bora strain, Santiago island, Cameroon and Malaysia (18). Although *Bti* resistance is apparently absent in *Ae. aegypti* populations to date, resistance has detected in *Culex pipiens*, from Syracuse, New York which had a resistance ratio of 33-fold when compared to the S-Lab susceptible strain (26). Resistance to *Bti* has also been demonstrated in *Aedes rusticus Rossi* mosquitoes, selected for resistance through annual *Bti* treatment in larval sites in the Rhône-Alpes region. The mosquitoes collected in the treatment area had a moderate resistance ratio up to 7.9 fold compared to the untreated area (27). The attained resistance levels were still relatively low compared to when mosquitoes are selected for resistance to other insecticides (27). The multiple active toxins-Cry4A, Cry4B, Cry11A and Cyt1A- produced by *Bti* might act at different receptors, making evolution of resistance to *Bti* very difficult (28). Nevertheless, both Saudi populations showed a significantly higher LC_{50} value than the New Orleans strain, suggesting that, as with temephos, variation exists which might be selected to higher levels in future.

Therefore, although the *Ae. aegypti* population in Jeddah and Makkah are still susceptible to temephos, rotational application of *Bti* and temephos, or another larvicide to which there is full susceptibility, would be advisable to slow down evolution of resistance to either of them thus retaining their efficacy over extended periods of use in vector control. Our findings suggest the potential to develop resistance to both insecticides may exist and thus mixture or rotation is advisable, along with continued monitoring, and consideration of other options such as insecticide growth regulators.

Conclusion

Aedes aegypti from Makkah and Jeddah remain susceptible to the larvicides assessed in this study and thus larval source management and larviciding could remain an effective tool in control, but regular monitoring and consideration of additional alternatives is advised.

Authors' contributions

AMA-N collected the field samples, performed the larval bioassays, analysed data and drafted the manuscript. SA conducted the larval bioassays and analysed data. DW conceived and designed the experiments, drafted the manuscript and analysed data. All authors read and approved the final manuscript.

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Competing interests

The authors declare no conflicts of interest.

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Table 1. Number of *Ae. aegypti* larvae from Jeddah and Makkah used in each bioassay at different times.

Temephos assay		*Bacillus thuringiensis israelensis* assay	
Strain	Sample size	Strain	Sample size
New Orleans	900	New Orleans	650
Makkah lab strain	900	Makkah lab strain	590
Jeddah lab strain	900	Jeddah lab strain	650
		Makkah field strain	546
		Jeddah field strain	567
		Cayman	605

Table 2. Temephos stock dilution with distilled water up to 200 ml to obtain the appropriate final concentrations.

Final concentration (mg/L)	Volume of stock solution (mL)	Volume of distilled water (mL)
0.08	1	199
0.07	0.875	199.125
0.06	0.75	199.25
0.04	0.5	199.5
0.03	0.375	199.625
0.02	0.25	199.75
0.01	0.125	199.875
0.005	0.0625	199.9375
0.0025	0.03125	199.96875

Table 3. *Bacillus thuringiensis israelensis (Bti)* stock dilution with distilled water from the stock solution at 1.2%.

C1 (stock) ppm	Volume of stock solution (mL)	Desired concentration (100mL)-ppm	Volume 2 (mL)
120	0.005	0.0059997	100.005
120	0.003	0.003599892	100.003
120	0.002	0.002399952	100.002
120	0.001	0.001199988	100.001
120	0.00075	0.000899993	100.00075
120	0.0005	0.000599997	100.0005

120	0.0002	0.00024	100.0002
120	0.0001	0.00012	100.0001

Table 4. Average percentage mortality of *Ae. aegypti* larvae from Makkah and Jeddah and the susceptible strain, New Orleans exposed to nine concentrations of temephos.

Strain	Temephos Concentration (mg/L)								
	0.0025 (%)	0.005 (%)	0.01 (%)	0.02 (%)	0.03 (%)	0.04 (%)	0.06 (%)	0.07 (%)	0.08 (%)
Makkah	0	0	9.18	70	89.6	100	99	99	100
Jeddah	1	0	1.02	32	79	73	94.8	98	100
New Orleans	5.2	9.9	52.6	81	96	99	100	100	100

Table 5. Average percentage mortality of *Ae. aegypti* larvae from Makkah, Jeddah (field and lab strains), New Orleans and Cayman strains exposed to eight concentrations of *Bti*.

	Bacillus thuringiensis israelensis (*Bti)* Concentration (ppm)							
Strain	0.00012 (%)	0.00024 (%)	0.0006 (%)	0.00089 (%)	0.0012 (%)	0.002 (%)	0.0036 (%)	0.006 (%)
Makkah field	10	19.3	26.2	58.1	63.2	98.8	98.4	100
Jeddah field	0	0	16.4	48.6	59.2	94.9	97.5	100
Jeddah lab	0	5	12.7	21.9	32.2	72.9	93.6	100
New Orleans	20.5	22.9	59.5	70	98.9	100	100	100
Cayman	5.3	12.5	21.6	43.1	52.2	95	100	100

Table 6. Lethal concentrations of temephos and *Bti* that kills 50% of *Ae. aegypti* strains.

	Temephos assay		*Bti* assay	
Strain	LC_{50}, mg/L (95% C.I.)	RR	LC_{50}, ppm (95% C.I.)	RR
New Orleans	0.010[a] (0.009-0.011)	1	0.00041[a] (0.000276-0.000537)	1
Makkah lab	0.017[b] (0.014-0.019)	1.7	n/a	n/a
Jeddah lab	0.029[c] (0.025-0.034)	2.9	0.001483[b] (0.001341-0.001629)	3.6
Makkah field	n/a		0.000834[c] (0.000688-0.000982)	2.1
Jeddah field	n/a		0.00098[b,c] (0.000882-0.001076)	2.4
Cayman	n/a		0.001018[b,c] (0.000882-0.001157)	2.5

Shared letters within a column indicate no significant difference based on overlapping confidence limits. The Makkah lab *Bti* assay was not calculated (n/c) because a very poor fit of the probit model meant that LC_{50} confidence intervals could not be reliably estimated even with a correction factor. Makkah and Jeddah field strains were not assessed (n/a) with temephos because the strains were not available at LSTM. The RR shown in the table indicates the resistance ratio.

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Table 1. Number of *Ae. aegypti* larvae from Jeddah and Makkah used in each bioassay at different times.

Temephos assay		*Bacillus thuringiensis israelensis* assay	
Strain	Sample size	Strain	Sample size
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Cayman	n/a		0.001018[b,c] (0.000882-0.001157)	2.5

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Key words: Mosquito larvae, Larval bioassay, *Bti*, temephos

Introduction

In Saudi Arabia, insecticides are extensively used to combat mosquito-borne diseases and other household pests, as well as in agriculture (Aziz et al.,2014). *Aedes aegypti* is primarily controlled by larvicides such as Spinosad (Natular®), *Bacillus thuringiensis israelensis* (*Bti*) toxin (VectoBac®), pyriproxyfen and diflubenzuron. Adulticides such as deltamethrin, permethrin, cyfluthrin and fenitrothion are also used for fogging and indoor residual spraying to reduce the density of adult mosquitoes during outbreak situations (World Health Organization, 1997). Temephos, *Bti*, Spinosad and insect growth regulatory hormones such as pyriproxyfen are used as larvicides in breeding sites, but *Bti* and Spinosad are more common in Jeddah and Makkah (Aziz et al.,2014, Mahyoub et al., 2013, Alsheikh et al., 2016). However, the extensive use of chemical insecticides has led to the development of insecticide resistance in *Ae. aegypti* worldwide including Saudi Arabia (Yaicharoen et al., 2005, Ranson et al., 2010, Marcombe et al., 2009, Jirakanjanakit et al., 2014, Rodríguez et al., 2005, Al Nazawi et al., 2017, Aziz et al., 2011, Alsheikh et al., 2016). In 2011, *Ae. aegypti* strains from Makkah were found to be resistant to lambda-cyhalothrin, deltamethrin, permethrin, bendiocarb and cyfluthrin (Aziz et al., 2011, Al Nazawi et al., 2017) but still susceptible to pirimiphos-methyl (actellic) and *Bacillus thuringiensis israelensis Bti* (Bacilod) (Aziz et al., 2011). In addition, Jeddah strains showed high prevalence of resistance to the pyrethroid deltamethrin and permethrin and the carbamate bendiocarb (Al Nazawi et al., 2017) but no studies to date have been considered larvicides. In Jazan, the population was resistant to lambda-cyhalothrin, DDT, bendiocarb and showed moderate resistance to permethrin, deltamethrin and fenitrothion (yet remained susceptible to cyfluthrin) (Alsheikh et al., 2016). The larvae were reported as highly resistant to temephos, but the documented LC_{50} of 61.8 mg/L, appears unfeasibly high being far beyond the LC_{50} reported for other temephos resistant populations in the world (Biber et al., 2006, dos Santos Dias et al., 2017) suggesting further investigation is essential.

A major limitation of the control program in the region is the limited surveillance to monitor the effectiveness of control intervention, or changes in the resistance of populations that may undermine the control efforts. We therefore assess the susceptibility status of the sole local dengue vector *Ae. aegypti* collected from Makkah of Saudi Arabia to larvicides (temephos and *Bti*). The outcome of this study will provide reliable, updated data on the resistance profile of larval *Ae. aegypti* populations from Saudi Arabia and may provide indication of which insecticides may be more effective.

Materials and Methods

Mosquito strain

Aedes aegypti larvae were collected from multiple breeding sites in two dengue endemic areas in Makkah (Lab= 21°45'2.13 N, 39°92'1.96 E; field=21°40'7.70 N, 39°86'3.19 E) and Jeddah (Lab=21°35'2.13 N,

39°13'9.42 E; field=21°60'3.97 N, 39°27'2.49 E). The lab strains were fifth generation from the original field which was collected in 03-04/2016(Al Nazawi et al., 2017). The field strain was collected in 01-02/2018. The larvae were reared to adults under insectary conditions of 27 ± 2 °C, 75% ± 10% R.H and L12:D12 photoperiod as described by (Al Nazawi et al., 2017). Two reference strains, Cayman, a multiply resistant lab strain, though reported as lacking temephos resistance (Harris et al., 2010), and the standard (ubiquitously-susceptible) strain New Orleans were used. All strains were raised under the same standard insectary conditions at the Liverpool School of Tropical Medicine (Al Nazawi et al., 2017).

Larval Bioassays

Larval bioassays were carried out on *Aedes* strains shown in **Table 1** according to the WHO protocol (World Health Organization, 2005) to determine the lethal concentrations (LC_{50}) and the resistance ratio relative to New Orleans (RR_{50}).

<Table 1>

Bioassays were performed using temephos (Sigma-Aldrich, Dorset, UK), or *Bti* (Vectobac®12AS 1.2%,1200 ITU/mg. A total of eight different concentrations of *Bti* and nine of temephos were used for each strain **(Table 2&3)**. The concentrations were selected as they have been reported to result in larval mortality between 10% and 95% (World Health Organization, 2005). The data were used to calculate the lethal dose that kills 50% (LC_{50}) in each population. Dilutions of temephos (stock dissolved in absolute ethanol) with distilled water up to a total volume of 200mL are detailed in **Table 2.** For each concentration of each insecticide, three or four replicates of a pool of approximately 25 late third or early fourth instar larvae were tested along with a negative control pool;1mL absolute ethanol mixed into 199 mL of distilled water for temephos or into 100ml of distilled water for *Bti* assays.

<Table 2>

<Table 3>

Vectobac stock (1.2%) was diluted by adding 1ml of the stock (1.2%) to 99 ml distilled water to obtain 0.012% (120pmm) which was used in the experiment.

All larval bioassays were performed in 6 cm in diameter plastic bowls; Mortality was recorded after 24h of exposure. Any larvae failing or unable to swim up to the surface independently were counted as dead. Any larvae that had pupated during exposure were omitted from the total count.

Statistical analysis

The mortality (%) was calculated for the number of mosquitoes or larvae that were dead after 24h exposure. The LC_{50} value for the larval bioassays was calculated using probit regression analysis (SPSS version 24) (Finney,1971). If chisq >0.05, confidence limits were adjusted accordingly (SPSS does this unless the fit is terrible, if it is it will not calculated Cis) (Finney,1971). The resistance ratio (RR) was calculated by comparison of the resistant Makkah and Jeddah strains against the susceptible New Orleans strain using the formula below to monitor the level of insecticide resistance in a field population.

$$\text{Resistance ratio (RR)} = \frac{LC_{50} \text{ of resistant strain}}{LC_{50} \text{ of susceptible strain}}$$

Results

Larval bioassays

Mortality was not observed in any strain in the control assays. Based on the mortality rate across different concentrations of temephos and *Bti*, resistance to the larvicides was higher in field strains when compared to the New Orleans strain (**Table 4 and Table 5**).

<Table 4>

<Table 5>

Indeed in both the temephos bioassays, the LC_{50} confidence intervals were not overlapping in comparisons of any strain, indicating a significant difference in mortality between the strains (**Table 6)**. However, whilst there is significant variation in susceptibility, current guidelines (Mazzarri and Georghiou, 1995), suggest that a resistance ratio <5 indicates limited/no resistance; 5-10 moderate resistance, and >10 is substantial resistance. Therefore, based on this classification, no definitive resistance to temephos and *Bti* was identified in any of the strains tested.

<Table 6>

Discussion

The current study was conducted to assess the susceptibility of larval *Ae. aegypti* to commonly used insecticides in the cities of Jeddah and Makkah. Larval bioassays did not detect resistance in either Makkah or Jeddah to temephos or *Bti* (all resistance ratios <5 compared to a standard susceptible strain (World Health Organization, 2016). In contrast, extreme temephos resistance in *Ae. aegypti* larvae from Jazan (LC_{50}=61.8 mg/L) was reported in 2016 (Alsheikh et al., 2016). When compared to the average LC_{50} of multiple separate studies of the susceptible reference strains Rockefeller, New Orleans and Bora Bora (Moyes et al., 2017), this equates to a resistance ratio above 10,000, far exceeding the highest ratio of 224 previously recorded (in Brazil; (Moyes et al., 2017)). This estimate from Jazan thus appears unlikely to be correct, and in the absence of additional data, a provisional assessment of temephos susceptibility in Saudi Arabia seems appropriate.

Temephos resistance in *Ae. aegypti* larvae has been recorded globally including British Virgin Islands (Wirth and Georghiou, 1999), Thailand (Ponlawat et al., 2005), Brazil (Melo-Santos et al., 2010), Cuba (Bisset et al., 2011), Colombia (Grisales et al., 2013), Martinique (Marcombe et al., 2012) and Santiago island (Rocha et al., 2015). Whilst the current data suggest susceptibility, it is important to note that both Saudi Arabian strains showed significantly higher LC_{50} values than the susceptible New Orleans strain.

Bacillus thuringiensis israelensis (*Bti*) is a bacterial derived toxin that has been widely used for vector control. The populations from Jeddah and Makkah were susceptible to this compound in comparison (based on a resistance ratio <5) with the New Orleans strain. Almost all other studies have reported similar findings, including Martinique populations (highly resistant to most insecticides) that were susceptible to *Bti* compared to the Bora-Bora strain, Santiago island, Cameroon and Malaysia (Moyes et al., 2017). Although *Bti* resistance is apparently absent in *Ae. aegypti* populations to date, resistance has detected in *Culex pipiens,* from Syracuse, New York which had a resistance ratio of 33-fold when compared to the S-Lab susceptible strain (Paul et al., 2005). Resistance to *Bti* has also been demonstrated in *Aedes rusticus Rossi* mosquitoes, selected for resistance through annual *Bti* treatment in larval sites in the Rhône-Alpes region. The mosquitoes collected in the treatment area had a moderate resistance ratio up to 7.9 fold compared to the untreated area (Boyer et al., 2012). The attained resistance levels were still relatively low compared to when mosquitoes are selected for resistance to other insecticides (Boyer et al., 2012). The multiple active toxins-Cry4A, Cry4B, Cry11A and Cyt1A- produced by *Bti* might act at different receptors, making evolution of resistance to *Bti* very difficult (Wirth, 2013). Nevertheless, both Saudi populations showed a significantly higher LC_{50} value than the New Orleans strain, suggesting that, as with temephos, variation exists which might be selected to higher levels in future.

Therefore, although the *Ae. aegypti* population in Jeddah and Makkah are still susceptible to temephos, rotational application of *Bti* and temephos, or another larvicide to which there is full susceptibility, would be advisable to slow down evolution of resistance to either of them thus retaining their efficacy over extended periods of use in vector control. Our findings suggest the potential to develop resistance to both insecticides may exist and thus mixture or rotation is advisable, along with continued monitoring, and consideration of other options such as insecticide growth regulators.

Conclusion

Aedes aegypti from Makkah and Jeddah remain susceptible to the larvicides assessed in this study and thus larval source management and larviciding could remain an effective tool in control, but regular monitoring and consideration of additional alternatives is advised.

Authors' contributions

AMA-N collected the field samples, performed the larval bioassays, analysed data and drafted the manuscript. SA conducted the larval bioassays and analysed data. DW conceived and designed the experiments, drafted the manuscript and analysed data. All authors read and approved the final manuscript.

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Competing interests

The authors declare no conflicts of interest.

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Table 1. Number of *Ae. aegypti* larvae from Jeddah and Makkah used in each bioassay at different times.

Temephos assay		*Bacillus thuringiensis israelensis* assay	
Strain	Sample size	Strain	Sample size
New Orleans	900	New Orleans	650
Makkah lab strain	900	Makkah lab strain	590
Jeddah lab strain	900	Jeddah lab strain	650
		Makkah field strain	546
		Jeddah field strain	567
		Cayman	605

Table 2. Temephos stock dilution with distilled water up to 200 ml to obtain the appropriate final concentrations.

Final concentration (mg/L)	Volume of stock solution (mL)	Volume of distilled water (mL)
0.08	1	199
0.07	0.875	199.125
0.06	0.75	199.25
0.04	0.5	199.5
0.03	0.375	199.625
0.02	0.25	199.75
0.01	0.125	199.875
0.005	0.0625	199.9375
0.0025	0.03125	199.96875

Table 3. *Bacillus thuringiensis israelensis (Bti)* stock dilution with distilled water from the stock solution at 1.2%.

C1 (stock) ppm	Volume of stock solution (mL)	Desired concentration (100mL)-ppm	Volume 2 (mL)
120	0.005	0.0059997	100.005
120	0.003	0.003599892	100.003
120	0.002	0.002399952	100.002
120	0.001	0.001199988	100.001
120	0.00075	0.000899993	100.00075
120	0.0005	0.000599997	100.0005
120	0.0002	0.00024	100.0002
120	0.0001	0.00012	100.0001

Table 4. Average percentage mortality of *Ae. aegypti* larvae from Makkah and Jeddah and the susceptible strain, New Orleans exposed to nine concentrations of temephos.

Strain	Temephos Concentration (mg/L)								
	0.0025 (%)	0.005 (%)	0.01 (%)	0.02 (%)	0.03 (%)	0.04 (%)	0.06 (%)	0.07 (%)	0.08 (%)
Makkah	0	0	9.18	70	89.6	100	99	99	100
Jeddah	1	0	1.02	32	79	73	94.8	98	100
New Orleans	5.2	9.9	52.6	81	96	99	100	100	100

Table 5. Average percentage mortality of *Ae. aegypti* larvae from Makkah, Jeddah (field and lab strains), New Orleans and Cayman strains exposed to eight concentrations of *Bti*.

	Bacillus thuringiensis israelensis (*Bti*) Concentration (ppm)							
Strain	0.00012 (%)	0.00024 (%)	0.0006 (%)	0.00089 (%)	0.0012 (%)	0.002 (%)	0.0036 (%)	0.006 (%)
Makkah field	10	19.3	26.2	58.1	63.2	98.8	98.4	100
Jeddah field	0	0	16.4	48.6	59.2	94.9	97.5	100
Jeddah lab	0	5	12.7	21.9	32.2	72.9	93.6	100
New Orleans	20.5	22.9	59.5	70	98.9	100	100	100
Cayman	5.3	12.5	21.6	43.1	52.2	95	100	100

Table 6. Lethal concentrations of temephos and *Bti* that kills 50% of *Ae. aegypti* strains.

	Temephos assay		*Bti* assay	
Strain	LC_{50}, mg/L (95% C.I.)	RR	LC_{50}, ppm (95% C.I.)	RR
New Orleans	0.010[a] (0.009-0.011)	1	0.00041[a] (0.000276-0.000537)	1
Makkah lab	0.017[b] (0.014-0.019)	1.7	n/a	n/a
Jeddah lab	0.029[c] (0.025-0.034)	2.9	0.001483[b] (0.001341-0.001629)	3.6

Makkah field	n/a		0.000834[c] (0.000688-0.000982)	2.1
Jeddah field	n/a		0.00098[b,c] (0.000882-0.001076)	2.4
Cayman	n/a		0.001018[b,c] (0.000882-0.001157)	2.5

Shared letters within a column indicate no significant difference based on overlapping confidence limits. The Makkah lab *Bti* assay was not calculated (n/c) because a very poor fit of the probit model meant that LC_{50} confidence intervals could not be reliably estimated even with a correction factor. Makkah and Jeddah field strains were not assessed (n/a) with temephos because the strains were not available at LSTM. The RR shown in the table indicates the resistance ratio.