Saudi Journal of Biological Sciences 30 (2023) 103581

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Biological comparative study between *Wolbachia*-infected *Aedes aegypti* mosquito and *Wolbachia*-uninfected strain, Jeddah city, Saudi Arabia

Abdullah G. Algamdi [a], Fekri M. Shaher [b,*], Jazem A. Mahyoub [a]

[a] Department of Biology Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[b] Hodeidah University, Hodeidah, Republic of Yemen

ARTICLE INFO

Article history:
Received 24 November 2022
Revised 6 January 2023
Accepted 29 January 2023
Available online 2 February 2023

Keywords:
Aedes aegypti
Wolbachia
Pesticide detoxification enzymes
Biological control
Pesticide resistance
Jeddah city

ABSTRACT

In this study, samples of *Wolbachia*-infected *Aedes aegypti* mosquitoes were collected from Al-Safa district in Jeddah city, Saudi Arabia. The presence of *Wolbachia* bacteria in mosquitoes was confirmed by PCR technique and they were reared and propagated in the laboratory. Comparative studies were conducted between *Wolbachia*-infected *A. Aegypti* and the *Wolbachia*-uninfected laboratory strain in terms of their ability to withstand drought, resist two types of insecticides and the activities of pesticide detoxification enzymes. The Wolbachia-infected *A. aegypti* strain proved less able to withstand the drought period, as the egg-hatching rate of the Wolbachia-uninfected strain was greater than that of the Wolbachia-infected strain after one, two and three months of dry periods. Compared to the *Wolbachia*-uninfected strain, the *Wolbachia*-infected strain demonstrated a relatively greater resistance to tested pesticides, namely Baton 100EC and Fendure 25EC which may be attributed to the higher levels of the detoxification enzymes glutathione-S-transferase and catalase and the lower levels of esterase and acetylcholine esterase.

© 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Dengue fever is a rapidly spreading disease in recent decades, especially in tropical and subtropical countries of the world. It is a viral disease transmitted by *Aedes aegypti* mosquito, and it has caused health and economic burdens on the countries where it is spread. The number of people who are susceptible to dengue fever is estimated at approximately 390 million annually (Guzman and Istúriz, 2010, Bhatt et al., 2013, Undurraga et al., 2013; WHO, 2019).

Currently, the method used to prevent the spread of the dengue virus is to control the mosquito vector using chemical pesticides (WHO, 2019). However, this method has proven to be insufficient for sustainable control of the disease (Bowman et al., 2016, WHO, 2019). Therefore there is an urgent need to devise new methods of mosquito combat and disease control. Several vector control methods are in the process of research and development to meet the need for sustainable control (Yakob and Walker, 2016, Von Seidlein et al., 2017).

Currently, a promising technology has emerged that can play a major role in controlling dengue disease, it depends on the development of mosquitoes that have the ability to resist the virus and prevent its spread.

This control technique is based on the use of *Wolbachia* bacteria, which have the ability to inhibit the dengue virus inside the host (Ferguson et al., 2015). *Wolbachia*-infected mosquitoes are reared in the laboratory and then released into the field, where they mate with wild mosquito populations, *Wolbachia* transmission occurs across generations due to cytoplasmic incompatibility (CI), and the percentage of infected mosquitoes increases to become the dominant mosquito strain in the target area (Walker et al., 2011). This experiment has been conducted in many countries and the result were promising successful (Hoffmann et al., 2011; Frentiu et al., 2014 Schmidt et al., 2017; O'Neill et al., 2018; Zheng et al., 2019; Zhang and Lui, 2020). The *Wolbachia* strategy also inhibits other viruses within the mosquito including Zika, yellow fever and chikungunya viruses (Van den Hurk et al., 2012; Dutra et al., 2016).

Wolbachia is naturally found in approximately 60 % of all insect species (Werren, 2008; Ferguson, 2015) and it is present naturally

* Corresponding author.
E-mail addresses: Agalghamdi1@kau.edu.sa (A.G. Algamdi), fshaher0001@stu.kau.edu.sa (F.M. Shaher), jabdulrahman@kau.edu.sa (J.A. Mahyoub).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2023.103581
1319-562X/© 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

in some types of mosquitoes, but it is not present in *A. aegypti* mosquitoes. One of the characteristics of *Wolbachia* is that when infected females mate with infected or uninfected males, the resulting eggs hatch, but when infected males mate with uninfected females, the resulting eggs do not hatch, and this is known as the cytoplasmic incompatibility (CI) phenomenon. In general, *Wolbachia*-infected mosquitoes produce fewer eggs and have shorter lifespan than uninfected strains (Zhang and Lui, 2020).

This study was designed to investigate some biological characteristics of *Wolbachia*-infected *A. aegypti* mosquitoes and compare them with those of the *Wolbachia*-uninfected strain including the following: the ability of eggs to withstand drought, sensitivity level to some pesticides used in control programs and the activity of detoxification enzymes as well as assessment of the interbreeding success between the two strains.

2. Methodology

2.1. Collecting Wolbachia-infected A. aegypti mosquitoes

A random group of sites in Al-Safa district, Jeddah, Saudi Arabia were selected to collect field strains of *A. aegypti* mosquitoes, where the Jeddah Municipality, in cooperation with King Abdulaziz City for Science and Technology (KACST), had previously released large numbers of *Wolbachia*-infected *A. aegypti* mosquitoes. BG-Sentinel mosquito traps were used for this purpose. The traps were supplied with lactic acid to attract mosquitoes. The coordinates of these locations (Fig. 1) were determined using the Global Positioning System (GPS) (Garmin International, Inc., 1200 E, Olathe, KS, 66062, USA). The contents of each trap were emptied into the adult mosquito cage and each trap was given a special code number. The mosquitoes were fed a blood meal using an artificial feeding device, Hemotek Membrane Feeding System.

The breeding cages contained small plastic cups containing water. The inner surface was lined with two filter papers (9 cm in diameter) on which mosquito eggs were placed. Filter paper simulates the favoured environment of *A. aegypti*, as this type of mosquito prefers to lay eggs on moist clay soils. After the eggs were laid, the filter papers were placed in larval culture dishes containing water, where the eggs hatched to larvae. After the emergence of the insects, they were analysed for the presence of *Wolbachia* bacteria using PCR. The *Wolbachia*-positive samples were reared to obtain enough numbers of larvae and adults to carry out the required experiments.

2.2. Detection of Wolbachia bacteria in A. aegypti mosquitoes

Each independent sample was crushed in BAPS buffer solution (Bovine Albumin Phosphate Saline) to obtain a suspension to be detected. DNA was extracted according to the method of Sarwar et al., (2022). A group of primers for *Wolbachia* bacteria identification was added to the rest of the reaction chemicals (Table 1). The mixture was prepared in special packages, and the heat cycles were programmed on the Thermocycler, where the polymerase chain reaction takes place. After the end of the thermal cycle, the agarose gel was prepared, and the RT-PCR products were subjected to thermal separation through the agarose gel. Then the samples were loaded onto the gel with a 100 bp DNA ladder marker, and electricity was generated using an electrophoresis device for nucleic acids. The location of the bunds on the gel was determined using UV light, and the bands were photographed by the gel documentation system (Carvajal et al., 2019; Teo et al., 2017).

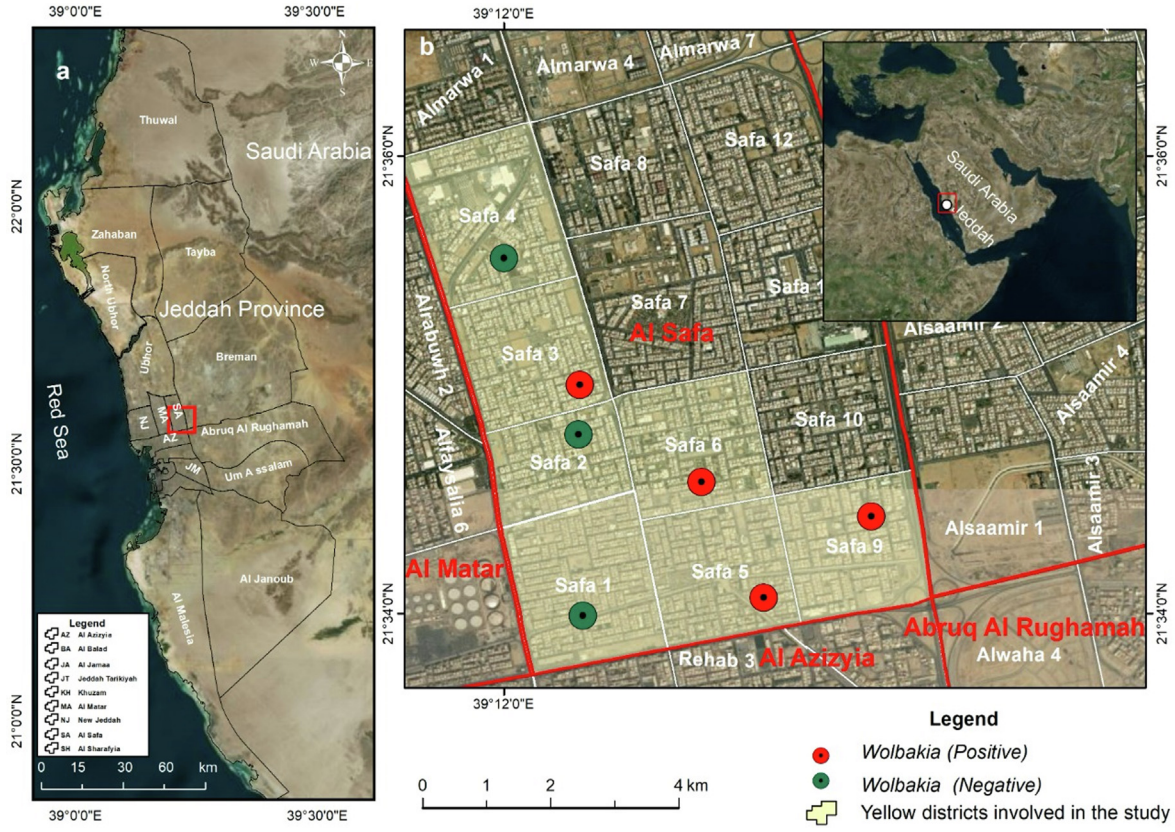


Fig. 1. Locality sites: sampling was carried out by installing the BG-Sentinel: Biogents' mosquito trap in various habitats of the Al-Safa district.

Table 1
The details of gene primers used for the detection and characterization of Wolbachia from *Ae. aegypti* collected from Al-Safa district.

Primers pair 5′-3′	Annealing	Target gene	Estimated product size
wsp_F TGG TCC AAT AAG TGA TGA AGAAAC wsp_R AAAAATTAAACGCTACTCCA	59C	Wsp gene	610 bp

2.3. Testing the effect of dry periods on the hatching rate of eggs under laboratory conditions

In the entomological laboratory, eggs of two strains of *A. aegypti* mosquitoes were preserved, the first was *Wolbachia*-infected and the other was *Wolbachia*-uninfected, the eggs were kept on Whatman filter paper No. (1) at a temperature of 27 °C and a relative humidity of approximately 70 % for a period of 30, 60 and 90 days to determine the effect of the dry period on the fertility of both strains and compare them with a reference strain that laid fresh eggs.

2.4. Detection of insecticide detoxification enzymes in A. aegypti mosquitoes

The extract was prepared according to the method described by (Algamdi and jazem, 2022). One hundred milligrams of adult *A. aegypti* mosquitoes were crushed in 10 ml of sodium phosphate buffer at a concentration of 20 mmol and pH 7.5. The crude extract was separated by centrifugation at 6000 rpm for 30 min at 4 °C. The separated upper layer was defined as the crude extract and kept at a temperature of −15 °C until it was used for measuring enzyme activity according to the method described by (Ellman et al., 1961).

2.5. The susceptibility level of Wolbachia-infected A. aegypti strain to some pesticides compared to the Wolbachia-uninfected strain

The World Health Organization (WHO, 2005) method was used to estimate the sensitivity of *A. aegypti* larvae to two types of pesticides. The mosquito larvae at their early fourth instar were exposed to each insecticide at different concentrations. Experiments were carried out in 250 ml glass beakers containing 100 ml water. The depth of water in the cup was approximately 5 cm (WHO, 2005). In all sensitivity tests, five replicates/concentration were used, where each replicate contained 20 larvae, in addition to five replicates of the control. The larvae were given their food during the test. The number of dead larvae was recorded after 24 h of testing, the percentage of larval death was calculated for each insecticide, and the results were analysed statistically. Statistical parameters were extracted from the toxicity curves such as chi-square (X_2) values, slope function (S), LC_{50} values, fiducial limits and slopes, according to Mahyoub, (2021).

2.6. Tested insecticides

Two insecticides used in the control program in Saudi Arabia belonging to pyrethroid and organophosphorus groups (Table 2) were evaluated for their larvicidal efficacy against the *Wolbachia*-infected *A. aegypti* strain and the *Wolbachia*-uninfected *A. aegypti* strain.

3. Results

3.1. Detection of Wolbachia bacteria in the field strain

Samples of adult *A. aegypti* collected from different sites in Al-Safa district, Jeddah Governorate, were subjected to PCR experiments. The piece of DNA to be studied was multiplied using the polymerase chain reaction (PCR) technique. The isolated DNA piece was analysed using the electrophoresis technique with an agarose gel (1.5 %) to ensure its length and purity. A radiograph of an agarose gel (1.5 %) with bands of DNA stained with ethidium bromide (EtBr) was shown in Fig. 2. The first lane contained the DNA ladder. all lanes contained DNA samples isolated from *A. aegypti* mosquitoes; the gel electrophoresis procedure was performed to detect the presence of *Wolbachia* bacteria in those samples. The results showed that samples with code numbers 1, 3, 4 and 7 were positive for the PCR examination (*Wolbachia*-infected), as shown on the radiograph of the agarose gel (1.5 %), where the nucleic acid replicated by polymerase chain reaction had a cut-off length of approximately 600 base pairs. On the other hand, the samples with code numbers 2, 5, and 6 were negative (*Wolbachia*-uninfected).

The *Wolbachia*-positive samples were reared in the laboratory for several generations (reaching up to ten generations at the time of this study), and to ensure the transmission of bacteria from the parents to the resulting offspring in a vertical way, random samples were given code numbers from 1 to 4 and prepared for PCR examination in the same way as before. The results in (Fig. 3) show the presence of *Wolbachia* in all tested samples.

3.2. Assessment of the interbreeding success between Wolbachia-uninfected A. aegypti strain and Wolbachia-infected A. aegypti strain

In this experiment, 100 males of the laboratory Wolbachia-uninfected strain were combined with 100 females of a laboratory *Wolbachia*-infected strain in one cage and reared in the laboratory for several generations to evaluate the success of interbreeding between the two strains and the transmission of *Wolbachia* to offspring. Thirteen random samples were analysed using the polymerase chain reaction (PCR) technique. The results showed that all samples were *Wolbachia*-positive by PCR examination except for two samples of code numbers 2 and 11, which were negative (Fig. 4).

3.3. Hatching rate of Wolbachia-infected A. aegypti eggs that lived during dry periods under laboratory conditions compared to that of the uninfected strain

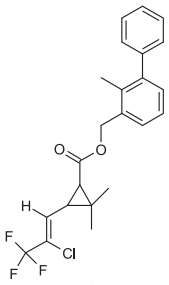
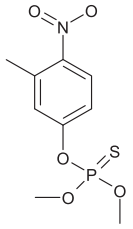
The results showed decreases in the hatching rate of *A. aegypti* eggs exposed to a thirty-day dry period of 45.8 and 38.1 %, a sixty-day dry period of 66.5 and 63.9 % and a ninety-day dry period of 87.0 and 84.5 % for the *Wolbachia*-infected and *Wolbachia*-uninfected strains, respectively. Meanwhile, the hatching rate decreased in freshly produced eggs by approximately 12 % (Table 3). Overall, the hatching rate decreased with increasing dry period.

3.4. Detection of the activity of insecticide detoxifying enzymes in the infected and uninfected-Wolbachia strains of A. aegypti

Studies have revealed that some groups of insects have become resistant to insecticides, and the mechanism of resistance varies according to different types of insects (Al Nazawi et al., 2017; Nancy et al., 2021).

Enzymes are used as biomarkers for evaluating the effects of toxins on exposed organisms (Badiou-Bénéteau et al., 2012;

Table 2
Tested insecticides against *Ae. aegypti* larvae.

	Pesticide name	Active ingredient	Molecular formula	Chemical structure	Group
1	Baton 100EC	Bifenthrin10% w/v	C23H22ClF3O2*		Pyrethroid
2	Fendure 25EC	Fenitrothion 50 %w/v	C9H12NO5PS**		Organophosphorus

*National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 5281872, Bifenthrin. Retrieved January 5, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Bifenthrin.
**National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 31200, Fenitrothion. Retrieved January 5, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Fenitrothion.

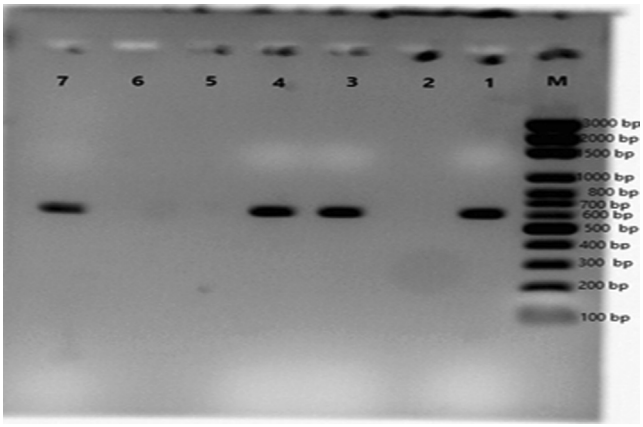


Fig. 2. Agarose gel electrophoresis of wsp gene in *Wolbachia* in *A. aegypti* mosquito M: Marker, samples (1–7).

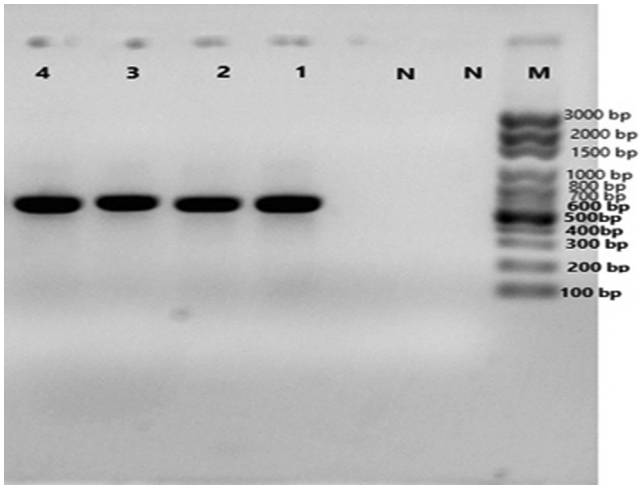


Fig. 3. Agarose gel electrophoresis of wsp gene in *Wolbachia* in the tenth generation of *A. aegypti* mosquito M: Marker N, negative control, samples (1,2,3 and 4).

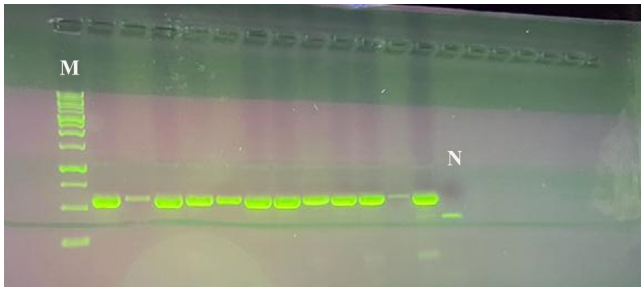


Fig. 4. Agarose gel electrophoresis of wsp gene in *Wolbachia* in *A. aegypti* mosquito M: Marker N, negative control, samples (1–13).

Carvalho et al., 2013; Almulaiky 2018). In this study, the activities of esterase, acetylcholine esterase (AChE), catalase, and glutathione- S-transferase enzymes in *A. aegypti* mosquitoes were estimated to investigate their sensitivity to the pesticides applied to them.

The activity levels of esterase in the *Wolbachia*-uninfected *A. aegypti* larvae control group (10.06 units/mg protein) were 1.55 and 1.17-fold higher than those of *Wolbachia*-uninfected *A. aegypti* larvae treated with Baton (6.49 units/mg protein), and Fendure (8.57 units/mg protein), respectively (Table. 4) however, the activity levels of esterase in the *Wolbachia*-infected *A. aegypti* larvae control group (8.02 units/mg protein) were 1.48 and 1.14-fold higher than those of *Wolbachia*-infected *A. aegypti* larvae treated with Baton (5.42 units/mg protein), and Fendure (7.01 units/mg protein), respectively (Table. 4). In the same manner the activity levels of (AChE) in the *Wolbachia*-uninfected *A. aegypti* larvae control (5.51 units/mg protein) were 1.28 and 1.70-fold higher than those of the *Wolbachia*-uninfected *A. aegypti* larvae treated with Baton (4.32 units/mg protein) and Fendure (3.24 units/mg protein), respectively. Additionally, the AChE activity level in the *Wolbachia*-infected *A. aegypti larvae* control group (3.55 units/mg protein) were 1.73 and 1.18-fold higher than those of the *Wolbachia*-

Table 3
The hatching rate of *A. aegypti* eggs after a dry period of 30,60 and 90 days under laboratory conditions.

Dry Periods (day)	No. of eggs used		No. of Larvae Hatched		Hatchability (%)[a]		Decrease in Hatchability (%)[b] Mean*±SE	
	infected	non infected	infected	non infected	infected	non infected	infected	non infected
30	50	50	21	24	47.7	54.5	45.8 ± 0.5[a]	38.1 ± 0.3[a]
60	50	50	13	14	29.5	31.8	66.5 ± 0.2[b]	63.9 ± 0.1[b]
90	50	50	5	6	11.4	13.6	87.0 ± 0.3[c]	84.5 ± 0.6[c]
Control	50		44		88			

*: *Means followed by the same letter in the same row are not significantly different according to LSD at α = 0.05.
a = $\frac{\text{No. of Larvae Hatched in treatment}}{\text{No. of Larvae Hatched in control}} \times 100$.

b = $\frac{\%\text{ hatchability in control} - \%\text{hatchability in treatment}}{\%\text{ hatchability in control}} \times 100$.

Table 4
Insecticide detoxification enzymes activities in *A. agypti* (infected and uninfected with *Wolbachia*).

Strain	Treatment	Enzyme activity (unit/mg protein)			
		Esterase	**Glutathione- S- Transferase**	**Catalase**	Acetylcholine esterase (AChE)
Ae. aegypti (*Wolbachia*-uninfected)	Control	10.06a ± 0.05	17.19c ± 0.128	153.54c ± 0.05	5.51a ± 0.15
	Baton	6.49c ± 0.36	58.34a ± 0.21	178.77a ± 0.29	4.32b ± 02
	Fendure	8.57b ± 0.29	36.18b ± 0.27	169.36b ± 0.51	3.24c ± 11
Ae. aegypti (*Wolbachia*-infected)	Control	8.02a ± 0.15	36.28c ± 0.25	159.28c ± 0.25	3.55a ± 0.05
	Baton	5.42c ± 09	68.22a ± 0.07	191.99a ± 0.20	2.05b ± 0.15
	Fendure	7.01b ± 05	53.29b ± 0.66	177.14b ± 0.18	1.63c ± 0.22
L S D		3.5	12.8	9.8	0.96
Pr > F		0.0001	0.0001	0.0001	0.0001

infected *A. aegypti* larvae treated with Baton (2.05 units/mg protein) and Fendure (1.63 units/mg protein), respectively.

In contrast, as shown in Table 4 for both *Wolbachia*-uninfected and *Wolbachia*-infected strains, higher catalase (CAT) and glutathione-S-transferase (GST) activities were observed in larvae treated with Baton and Fendure pesticides compared to their respective controls

3.5. Susceptibility level of the Wolbachia-infected A. aegypti strain to some pesticides compared to that of the uninfected strain

The results in Table. 5 show that the mortality percentages of the fourth instar larvae of *A. aegypti* mosquitoes treated with Baton and Fendure were directly proportional to the concentration. With Baton treatment, the mortality rate ranged from 29 to 98 % for the *Wolbachia*-uninfected strains and from 20 to 92 % for the Wolbachia-infected strain at concentrations of 0.02–0.20 ppm. Additionally, in the case of the Fendure compound, the mortality rate ranged from 17 to 98 % for the *Wolbachia*-uninfected strain and from 8 to 93 % for the *Wolbachia*-infected strain at concentrations of 0.02–0.2 ppm (Table 5).

Table 5
Susceptibility levels of *A. aegypti* mosquito larvae (Infected and uninfected with Wolbachia) to Baton and Fendure.

Compound Name	Effective Concentrations (ppm)	Larval mortality [a] (%) Mean ± SE	
	Effective	**uninfected *Ae. Aegypti***	**Infected *Ae. aegypti***
Baton	0.02	29e ± 1.75	20e ± 1.50
	0.04	56d ± 2.33	44d ± 1.33
	0.07	75c ± 3 0.35	58c ± 1.25
	0.1	90b ± 3.20	74b ± 1.75
	0.2	98a ± 3.75	92a ± 2.50
Fendure	0.02	17e ± 1.50	8e ± 0.50
	0.04	52d ± 1.35	33d ± 0.33
	0.07	77c ± 1.25	60c ± 1.20
	0.1	90b ± 1.75	83b ± 1.50
	0.2	98a ± 1.25	93a ± 1.50

a.Five replicates, 20 larvae each; Control mortalities ranged from 0.0 – 2%; Means followed by the same letter are not significantly different according to LSD at 0.05.
b. $\chi 2$ Tabulated at 0.05 probability level = 7.81.

By studying the toxicity lines (LC-P lines) (Fig. 5) and performing statistical analysis (Table 6), the LC_{50} and LC_{90} values for the pyrethroid pesticide Baton were 0.0353 and 0.1101 ppm for the *Wolbachia*-uninfected strain and 0.0516 and 0.1966 ppm for the *Wolbachia*-infected strain, respectively.

For the organophosphorus pesticide Fendure 25EC, the LC_{50} and LC_{90} values were 0.0407 and 0.104 ppm for the *Wolbachia*-uninfected strain and 0.0577 and 0.1491 ppm for the *Wolbachia*-infected strain, respectively (Table 6).

The obtained results and the resistance ratio (RR) confirm that the pyrethroid pesticide Baton was more effective than Fendure against the fourth instar larvae by approximately 1.11 and 1.15-fold for the *Wolbachia*-infected and *Wolbachia*-uninfected strains, respectively (Table 6). On the other hand, the *Wolbachia*-infected *A. aegypti* strain displayed more tolerance of the tested pesticides Baton and Fendure than the *Wolbachia*-uninfected strain by approximately 1.46 and 1.42-fold, respectively (Table 6 & Fig. 5).

4. Discussion

The *Wolbachia* positive results obtained for the PCR analysis confirmed the prevalence of *Wolbachia* bacteria in wild populations in Al-Safa district, Jeddah, Saudi Arabia and the vertical transmission of *Wolbachia* through generations. These findings, in addition to the success of the interbreeding experiment between the *Wolbachia*-uninfected *A. aegypti* strain and *Wolbachia*-infected *A. aegypti* strain, are consistent with many previous studies that have shown the transmission of *Wolbachia* bacteria from parents to offspring and that introduction of bacteria *Wolbachia* through the release of an infected strain is an effective method to prevent dengue infection (Dean et al., 2005; Khoo et al., 2005; Axford et al., 2016; Huang et al., 2017). The polymerase chain reaction (PCR) technique can be applied to detect microorganisms and pathogens in mosquitoes including *Wolbachia* bacteria (Hoffmann et al., 2011; Beckmann and Fallon, 2013).

Wolbachia-uninfected *A. aegypti* eggs proved to withstand drought more than *Wolbachia*-infected eggs, but the difference was not significant; however, significant differences in the rate of reduction of hatching with increasing dry period was observed for both strains compared to the control (newly laid eggs). In gen-

Table 6
Statistical parameters of *A. aegypti* against selected pesticides.

Compound Name	Statical Parameters							
	LC_{50} (ppm)		LC_{90} (ppm)		Slope		$(Chi)^2$	
	Non-Infected	Infected	Non-Infected	Infected	Non-Infected	Infected	Non-Infected	Infected
Baton	0.0353	0.0516	0.1101	0.196	2.5908	2.2124	1.2462	1.343
Fendure	0.0407	0.0577	0.104	0.1491	3.1461	3.112	0.5654	3.0724

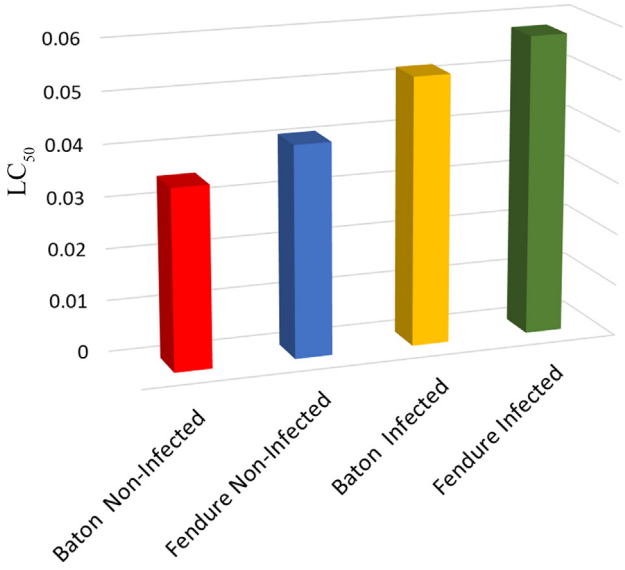


Fig. 5. LC_{50} values of tested pesticides against *Wolbachia*-infected and uninfected larvae of *A. aegypti*.

eral, the ability of *A. aegypti* eggs to withstand drought is one of the reasons for the growth and spread of dengue fever. A rapid outbreak of adult mosquitoes is always observed after 7–10 days of rain, which confirms that drought has no significant adverse effects on *A. aegypti* eggs and that the eggs retain their vitality in dry soils and re-hatch when exposed to water (Fischer et al., 2011; Stephanie et al., 2012; Alkuriji et al., 2020; Zhang and Lui, 2020).

Insects, including mosquitoes, have enzymes that degrade toxins called detoxification enzymes, many of which are enzymes in the cytochrome P450 system. These enzymes are responsible for degrading strange and toxic substances in insects as a defence system and a type of metabolic resistance.

Esterase is an important biomarker of organophosphorus and carbamate insecticides (Fourcy et al., 2002) and is used to assess the exposure of an organism to esterase inhibitor substances. Studies have shown that organophosphorus insecticide OPs have an inhibitory effect on the enzyme esterase (Fairbrother et al., 1989, Holmes and Boag, 1990). Similarly, AChE catalyses the hydrolysis of choline esters. Therefore, the inhibition of AChE by pesticides in aquatic animals is also considered biomointor for pollution (Rank et al., 2007; Ahmad et al., 2016).

Conversely, a high level of CAT activity was detected in aquatic animals exposed to pollution conditions. Therefore, CAT of fish is considered an important biomointor for the detection of pollution in water (Atli et al., 2006; Yu et al., 2011). In the same way, GST is involved in the detoxification of contaminated compounds. The level of GST activity increased in African catfish *Clarias gariepinus* in water polluted with heavy metals from the Ogun River (Nigeria) (Farombi et al., 2007).

The results in Table 4 show significant inhibition of esterase and AChE activities in the *Wolbachia*-infected *A. aegypti* strain by approximately 1.25 and 1.55-fold compared to the *Wolbachia*-uninfected *Ae aegypti* strain, respectively. In contrast, CAT and GST activities were significantly higher in the *Wolbachia*-infected *A. aegypti* strain by 1.04 and 2.11-fold compared to the *Wolbachia*-uninfected strain, respectively.

This can be explained by the possibility of an adaptive response of mosquitoes to the presence of *Wolbachia* bacteria which can generate enzymatic and physiological resistance to the bacteria.

The pesticide bioassay proved that the pyrethroid pesticide Baton was more effective against the fourth instar larvae of *A. aegypti* than the organophosphorus pesticide Fendure. This may be attributed to the different active groups as well as the mode of action of these two pesticides (Mahyoub et al.,2015 and 2016). On the other hand, the *Wolbachia*-infected *A. aegypti* strain displayed more tolerance to the tested pesticides Baton and Fendure than the *Wolbachia*-uninfected *A. aegypti* strain. This may be due to the higher activities of detoxification enzymes in the infected strain than in the uninfected strain. Therefore, it is reasonable to expect that the increase in enzyme activity induced by toxic substances could reduce the toxicity of the insecticide due to the increase in the detoxification process (Booth and O'Halloran, 2001, Badiou-Bénéteau et al., 2012).

5. Conclusion

The results obtained above indicated that there is a spread of *Wolbachia* bacteria in the Al-Safe district, Jeddah, Saudi Arabia where *A. aegypti* carrying *Wolbachia* were released by the Municipality of Jeddah Governorate in cooperation with King Abdulaziz City for Science and Technology.

Further studies are required to explore the prevalence of *Wolbachia* in the mosquito populations of other districts. The results showed that *Wolbachia*-infected mosquitoes were more tolerance to the tested pesticides compared to the uninfected strain, which requires conducting advanced studies to investigate whether it is due to the inducing factor (bacteria introduced to mosquitoes artificially) or because of genetics as a kind of pre-adaptation to acquire the characteristic of resistance against pesticides. Experiments are also required to investigate the ability of *Wolbachia* in controlling viruses that transmit viral hemorrhagic fevers endemic in the Kingdom, such as Rift Valley fever and chikungunya.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors express their great thanks and gratitude to the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia for the financial support of the research project under grant No. (G:180-130-1443).

References

Ahmad, S.A., Sabullah, M.K., Shamaan, N.A., Abd Shukor, M.Y., Jirangon, H., Khalid, A., Syed, M.A., 2016. Evaluation of acetylcholinesterase source from fish, Tor tambroides for detection of carbamate. J Environ Biol. 37 (4), 479–484.

Al Nazawi A. M., Aqili J., Alzahrani M., Philip J. M. and Weetman D. 2017. Combined target site (kdr) mutations play a primary role in highly pyrethroid resistant phenotypes of *Aedes aegypti* from Saudi Arabia. *Parasites Vectors*, 10:161. DOI 10.1186/s13071-017-2096-6.

Algamdi, G.A., Mahyoub, J.A., 2022. Detection of insecticide detoxification enzymes activities in *Aedes aegypti* mosquito, the vector of dengue fever in Saudi Arabia. Main Group Chemistry 21, 1053–1063. https://doi.org/10.3233/MGC-210184.

Alkuriji, M. A., Al-Fageeh, M. B., Shaher, F. M. and Almutairi B. F. 2020. Dengue Vector Control: A Review for Wolbachia-based Strategies, Biosci., Biotech. Res. Asia, 2020; 17(3): 507-515.

Almulaiky, Y. Q. 2018. Enzymes of Variola louti as potential biomarkers of aquatic pollution with a partial treated sewage. Cah. Biol. Mar, 59, 509-515.

Atli, G., Alptekin, O., Tükel, S., Canli, M., 2006. Response of catalase activity to Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ in five tissues of freshwater fish *Oreochromis niloticus*. Comp Biochem Physiol 143 (2), 218–224.

Axford, J. K., Callahan, A. G., Hoffmann, A. A., Yeap, H. L. and Ross, P. A. 2016. "Fitness of wAlbB Wolbachia infection in *Aedes aegypti*: parameter estimates in an outcrossed background and potential for population invasion," Am J of Trop Med Hyg, 94(3):507–516 View at: Publisher Site | Google Scholar.

Badiou-Bénéteau, A., Carvalho, S.M., Brunet, J.L., Carvalho, G.A., Buleté, A., Giroud, B., Belzunces, L.P., 2012. Development of biomarkers of exposure to xenobiotics in the honey bee *Apis mellifera*: application to the systemic insecticide thiamethoxam. Ecotoxicology and environmental safety 82, 22–31.

Beckmann J. F. 1 and Fallon, A. M. 2013. Decapitation Improves Detection of Wolbachia pipientis (Rickettsiales: Anaplasmataceae) in *Culex pipiens* (Diptera: Culicidae) Mosquitoes by the Polymerase Chain Reaction. J Med Entomol. 49(5): 1103-1108.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J. M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, G.R.W., Simmons, C.P., Scott, T.W., Farrar, J.J., Hay, S.I., 2013. The global distribution and burden of dengue. Nature 496, 504–507.

Booth, L.H., O'Halloran, K., 2001. A comparison of biomarker responses in the earthworm Aporrectodea caliginosa to the organophosphorus insecticides diazinon and chlorpyrifos. Environ. Toxicol. Chem. 20 (11), 2494–2502.

Bowman, L.R., Donegan, S., McCall, P.J., 2016. (2016) Is dengue vector control deficient in effectiveness or evidence?: systematic review and meta-analysis. PLoS Negl Trop Dis. 10, e0004551.

Carvajal, T.M., Hashimoto, K., Harnandika, R.K., Amalin, D.M., Watanabe, K., 2019. Detection of *Wolbachia* in field-collected *Aedes aegypti* mosquitoes in metropolitan Manila, Philippines. Parasites Vectors 12 (361).

Carvalho, S.M., Belzunces, L.P., Carvalho, G.A., Brunet, J.L., Badiou-Beneteau, A., 2013. Enzymatic biomarkers as tools to assess environmental quality: a case study of exposure of the honeybee *Apis mellifera* to insecticides. Environ toxicol chem 32 (9), 2117–2124.

Dean, Z. Xi, J. L. C. Khoo, and S. L. Dobson 2005. "Generation of a novel Wolbachia infection in *Aedes albopictus* (Asian tiger mosquito) via embryonic microinjection," Insect Biochem Mol Biol, 35 (8): 903–910.

Dutra, H.L.C., Rocha, M.N., Días, F.B.S., Mansur, S.B., Caragata, E.P., Moreira, L.A., 2016. Wolbachia blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. Cell Host Microbe. 19 (6), 771–774.

Ellman, G.L., Courtney, K.O., Andrres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7, 88–95.

Fairbrother, A., Bennet, R.S., Bennet, J.K., 1989. Sequential sampling of plasma cholinesterase in mallards (Anas platyrhynchos) as indicator of exposure to cholinesterase inhibitors. Environ Toxicol Chem 8, 117–122.

Farombi, E.O., Adelowo, O.A., Ajimoko, Y.R., 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (*Clarias gariepinus*) from Nigeria Ogun River. Int J of Environ Res Public Health 4, 158–165.

Ferguson, N. M., Kien, D. T. H., Clapham, H., Aguas, R., Vu, T. T., Chau, T. N. B., et al. 2015. Modeling the impact on virus transmission of wolbachia-mediated blocking of dengue virus infection of *Aedes aegypti*. Sci Transl Med, 7(279), 279ra37e279ra37.

Ferguson, N.M. 2015. Modeling the impact on virus transmission of Wolbachia-mediated blocking of dengue virus infection of *Aedes aegypti*. Sci. Transl. Med. 2015; 7:27937-27937.

Fischer, S., Alem, I.S., de Majo, M.S., Campos, R.E., Schweigmann, N., 2011. Cold season mortality and hatching behavior of *Aedes aegypti* L. (Diptera: Culicidae) eggs in Buenos Aires City. Argentina. *J Vector Ecol*. 36, 94–99. https://doi.org/10.1111/j.1948-7134.2011.00145.x.

Fourcy, D., Jumel, A., Heydorff, M., Lagadic, L., 2002. Esterases as biomarkers in Nereis (Hediste) diversicolor exposed to temephos and Bacillus thuringiensis var. israelensis used for mosquito control in coastal wetlands of Morbihan (Brittany, France). Mar environ res 54 (3–5), 755–759.

Frentiu, F.D., Zakir, T., Walker, T., Popovici, J., Alyssa, T.P., van den Hurk, A., et al., 2014. Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. PLoS Negl Trop Dis 8 (2), e2688.

Guzman, A., Istúriz, R.E., 2010. Update on the global spread of dengue. Int J Antimicrob Agents 36S, S40–S42.

Hoffmann, A.A., Montgomery, B.L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F., Greenfield, M., Durkan, M., Leong, Y.S., Dong, Y. et al. 2011. Successful establishment of Wolbachia in *Aedes* populations to suppress dengue transmission. Nature. 476:454–457. [PubMed] [Google Scholar].

Holmes, S.B., Boag, P.T., 1990. Inhibition of brain and plasma cholinesterase activity in Zebra finches orally dosed with fenitrothion. Environ Toxicol Chem 9, 323–334.

Huang, M.G.J.W., Luo, L.C.H., 2017. Assessing the efficiency of Wolbachia, driven, Aedes, mosquito suppression by delay differential equations. J Theor Biol 440, 1–11.

Khoo, Z., Xi, C.C., Dobson, S.L., 2005. Wolbachia establishment and invasion in an *Aedes aegypti* laboratory population. Science 310 (5746), 326–328.

Mahyoub, J.A., 2021. Bioactivity of two marine algae extracts and its synthesized silver nanomaterial against housefly, Musca domestica as a safety method for its control. Entomological Research 8, 1–8. https://doi.org/10.1111/1748-5967.12512.

Mahyoub, J.A., Alsobhi, A.S., Al-Ghamdi, K., Khatter, N.A., Al, T.A., Al-Shami, S.A., Panneerselvam, C., Murugan, K., Nicoletti, M., Canale, A., Benelli, G., 2016. Effectiveness of seven mosquito larvicides against the West Nile vector *Culex pipiens* (L.) in Saudi Arabia. *Asian Pac*. J Trop Biomed. 6 (5), 361–365.

Mahyoub, J. A., Al Thbiani, A., AL-Refaee, M., Rehman, H., Saggu, S., Murugan, K., Panneerselvam, C., Nicoletti, M., Canale, A., Benelli, G. 2015. Insecticide susceptibility in larval populations of the West Nile vector *Culex pipiens* L. (Diptera: Culicidae) in Saudi Arabia. Asian Pac J Trop Biomed., 6(5): 390-395.

Nancy M. Endersby–Harshman, AboElgasim A., Alhumrani B., Alkuriji M. A., Al–Fageeh M. B., Al–Malik A., Alsuabeyl M. S., Elfekih S., Hofmann A. A. 2021. Voltage–sensitive sodium channel (Vssc) mutations associated with pyrethroid insecticide resistance in *Aedes aegypti* (L.) from two districts of Jeddah, Kingdom of Saudi Arabia: baseline information for a Wolbachia release program. Parasites Vectors, 14:361:1-13.

O'Neill, S.L., Ryan, P.A., Turley, A.P., 2018. (2018) Scaled deployment of Wolbachia to protect the community from Aedes transmitted arboviruses. Gates Open Res. 2 (36), 1–28.

Rank, J., Lehtonen, K.K., Strand, J., Laursen, M., 2007. DNA damage, acetylcholinesterase activity and lysosomal stability in native and transplanted mussels (Mytilus edulis) in areas close to coastal chemical dumping sites in Denmark. Aquatic Toxicology 84, 50–61.

Sarwar, M.S., Jahan, N., Ali, A., Yousaf, H.K., Munzoor, I., 2022. Establishment of Wolbachia infection in *Aedes aegypti* from Pakistan via embryonic microinjection and semi-field evaluation of general fitness of resultant mosquito population. Parasites Vectors 15(1), 191, 1–13.

Schmidt, T.L., Barton, N.H., Rašić, G., 2017. Local introduction and heterogeneous spatial spread of dengue-suppressing Wolbachia through an urban population of *Aedes aegypti*. PLoS Biol. 15, e2001894.

Stephanie Margarete Thomas, Ulla Obermayr, Dominik Fischer, Juergen Kreyling & Carl Beierkuhnlein 2012. Low-temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, *Aedes albopictus* (Diptera: Culicidae). Parasites Vectors,5(100): 1-7

Teo, C.H.J., Lim, P.K.C.*, Voon, K. and Mak, J.W. 2017. Detection of dengue viruses and Wolbachia in Aedes aegypti and Aedes albopictus larvae from four urban localities in Kuala Lumpur, Malaysia. Tropical Biomedicine 34(3): 583–597.

Undurraga, E.A., Halasa, Y.A., Shepard, D.S., 2013. Use of expansion factors to estimate the burden of dengue in southeast Asia: a systematic analysis. PLoS Negl Trop Dis 7, e2056.

Van den Hurk, A.F., Hall-Mendelin, S., Pyke, A.T., et al., 2012. Impact of Wolbachia on infection with chikungunya and yellow fever viruses in the mosquito vector *Aedes aegypti*. PLoS Negl Trop Dis. 6, 892.

Von Seidlein, L., Kekulé, A.S., Strickman, D., 2017. Novel vector control approaches: the future for prevention of Zika virus transmission? PLoS Med. 14, e1002219.

Walker, T., Johnson, P.H., Moreira, L.A., et al., 2011. The wMel Wolbachia strain blocks dengue and invades caged Aedes aegypti populations. Nature. 476, 450–453.

Werren, J.H., 2008. Wolbachia master manipulators of invertebrate biology. Nat. Rev. Microbiol. 6, 741–751.

WHO, 2005. Prevention and control of dengue and dengue hemorrhagic fever, WHO, Regional Publication, 29, 134.

WHO, 2019. Dengue and severe dengue. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue.

Yakob, L., Walker, T., 2016. Zika virus outbreak in the Americas: the need for novel mosquito control methods. Lancet Glob Health. 4 (3), 148–149.

Yu, O., Vasylkiv, O., Kubrak, O.I., Storey, K.B., Volodymyr, I., Lushchak, V.I., 2011. Catalase activity as a potential vital biomarker of fish intoxication by the herbicide aminotriazole. J. PestBP 101, 1–5.

Zhang, H., Lui, R., 2020. Releasing Wolbachia-infected *Aedes aegypti* to prevent the spread of dengue virus: A mathematical study. Infect Dis Model 5, 142–160.

Zheng, X., Zhang, D., Li, Y., Yang, C., Wu, Y., Liang, X., Liang, Y., Pan, X., Hu, L., Sun, Q., Wang, X., 2019. Incompatible and sterile insect techniques combined eliminate mosquitoes. Nature 572 (7767), 56–61.