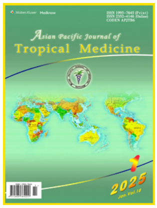

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Mosquito larval habitats, meteorological factors, and alternatives for vector control in Makkah Al-Mukarramah, the Kingdom of Saudi Arabia

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ABSTRACT

Objective: To characterize mosquito larval habitats and the influence of meteorological factors on their prevalence, and to suggest alternatives for vector control in Makkah Al-Mukarramah.

Methods: A mosquito survey was conducted within the 16 municipalities of Makkah Al-Mukarramah, from November 2022 to October 2023. The characteristics of larval habitats used by all reported species as oviposition sites were determined. Seasonal house, container, and breteau indices were used to determine larval abundance.

Results: 16 Species belonging to five genera [*Aedes* (3 spp.), *Anopheles* (3 spp.), *Culex* (8 spp.), *Culiseta* (1 sp.), and *Lutzia* (1 sp.)] were collected. A total of 185 608 potential mosquito larval habitats were surveyed. Of these, 95 853 (67.4%) were *Aedes*, 45 522 (32%) were *Culex*, 718 (0.5%) were *Anopheles*, and 38 (0.03%) were other species; 154 726 (83.4%) were water sources for mosquito larvae, and among these sources, 7 663 (5.0%) were positive for larvae, with 45.9% indoors and 54.1% outdoors. Most of the positive larval habitats were recorded in Al-Shawqiya (1 093, 14.3%), Al-Sharayia (1 003, 13.1%) and Al-Umrah (984, 12.8%). A total of 142 131 mosquito larvae and pupae were collected. The majority number of positive residences for all mosquito larvae was observed in January 2023 (1 658, 21.6%).

Conclusions: New appropriate alternatives for vector control are proposed, such as mechanical, biological, and environmental control.

KEYWORDS: *Aedes*; *Culex*; Dengue; Surveillance; Flora; Mechanical control; Environmental manipulation; Topology

1. Introduction

In recent years, the importance of vector-borne disease has increased globally[1]. Mosquitoes are important vectors of pathogens that cause diseases for humans and animals and are one of the most relevant groups of arthropods in public health[2,3]. The major vectors are *Aedes*, *Anopheles*, and *Culex* genera members. All these genera are found in the Kingdom of Saudi Arabia (KSA)[4].

Mosquitoes have diverse habitats that allow them to colonize various environments[5,6]. Thus, mosquitoes develop in a wide range of aquatic habitats. They occur primarily in temporary or permanent

Summary

Question: What are the mosquito larval habitats in Makkah Al-Mukarramah?

Findings: This survey of mosquito larval habitats across 16 municipalities revealed 185 608 potential habitats, with 83.4% identified as water sources, and a larval positivity rate of 5%. *Aedes* was the most common larvae species (67.4%).

Meaning: A combination of mechanical, biological, and environmental control strategies can significantly enhance vector control efforts.

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bodies of groundwater, but many species occupy leaf axils, tree holes, rock holes, crabholes, bamboo internodes, bromeliads and aroids, fruit shells and husks, fallen leaves and spathes, flower bracts, snail shells, and pitcher plants. Some utilize artificial containers and normal groundwater habitats[7]. The life cycle of mosquito is affected by both biotic and abiotic factors. Abiotic factors include the characteristics of larval habitats, surrounding vegetation, temperature, and rainfall[8]. The physical and chemical nature of the water determines the selection of the oviposition sites[9]. Understanding oviposition preference and ecological data, such as larval habitats, specific composition, and seasonal abundance, play a vital role in the management of mosquitoes, and neglecting to understand larval ecology can hamper control efforts. Control of the vector is dependent on characterizing these larval habitats[6]; therefore, studying larval habitats for different mosquito fauna will help to monitor potential modifications in larval habitats because of rainfalls, climate change, or man-made activities[8].

Several studies have reported on mosquito fauna and seasonal abundance in KSA, particularly in Makkah city in 2009-2010[10], Makkah, Al-Madinah and Tabuk in 2013-2014[9], and Makkah Al-Mukarramah in 2015[11]. A few studies were conducted in the eastern region in 2004-2006[12]. In addition, one study from Makkah has reported on clinical features during the first outbreak of dengue fever from April to July 2004[13], while a study from Jeddah has reported the factors associated with the spread of dengue fever[14]. Furthermore, Alahmed *et al.*[15] published an updated list of the mosquitoes of KSA, including 51 species under seven genera. Previous studies from adjacent countries have reported specific aspects of mosquito habitat and surveillance data, such as in Sudan[16], Ethiopia[17], Qatar[6], Iran[18], Libya[19], and Egypt[20].

The peculiar zoogeographic location and economic importance of KSA put it at potential risk of disease outbreaks. Numerous mosquito-borne pathogens cause many diseases within the country because of human practices[8]. Many residents are used to frequent water shortages and tend to store water in the home for extended periods. This practice has contributed to domestic water storage in various containers such as barrels, tanks, and cement tanks. These containers are usually uncovered and placed in shaded areas within house yards. Such practices have been often associated with increased mosquito productivity[10,11]. In KSA, the dengue virus was detected during the first dengue fever outbreak in Jeddah in 1994, and in Makkah in 2004. In the years 2020 to 2022, 9 443 confirmed cases have been reported in KSA (2 375, 3 421, and 3 647, respectively), while 1 725 confirmed cases have been recorded in Makkah (196, 1 146, and 383, respectively)[21]. According to the World Health Organization (WHO), vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700 000 deaths annually, mostly due to malaria and dengue fever[22].

Owing to the regular occurrence of dengue virus infection in Makkah, continued surveillance and effective vector control programs are warranted that are adapted to the unique population dynamics of Makkah, which receives millions of pilgrims annually from all over the world during the Hajj pilgrimage[23].

An epidemic of dengue fever could occur at any time in Makkah; however, vector control strategies are limited. Furthermore, information is lacking on mosquito larval habitats and the activity of vectors. In addition, to put in place appropriate prevention and control plans requires a better understanding of the population dynamics of Makkah and the identification of the areas at highest risk of disease outbreaks. Therefore, we characterized mosquito larval habitats and the influence of meteorological factors on the prevalence of mosquito in Makkah Al-Mukarramah, and then proposed appropriate alternatives for vector control.

2. Materials and methods

2.1. Study areas

KSA is divided into 13 provinces within five regions: northern, southern, central, eastern, and western. Makkah Province is in the western region of KSA and divided into 11 governorates, including Makkah Al-Mukarramah, which is the province's capital and the holiest city in Islam, as shown in Figure 1.

Figure 1. Map of Saudi Arabia showing Makkah Province including Makkah Al-Mukarramah.

Makkah city is a 70 km inland from Jeddah on the Red Sea, in a narrow valley 277 m above sea level and situated at 21° 30' N and 41° 00' E. In addition, Makkah is one of the most frequently visited cities in the world to perform Hajj and Umrah, where pilgrims more than triple the population number every year during the Hajj pilgrimage[23].

The focus of this study was Makkah Al-Mukarramah, which is divided into 16 municipalities within five sectors North (3), South (3), Central (4), East (3), and West (3): Al-Aziziya (AZ), Al-Hudaybiyah (HU), Al-Jumum (JM), Al-Ma'abdah (MA), Al-Masfalah (MS), Al-Otaybiya (OT), Al-Sharayia (SR), Al-Shawqiya (SQ), Al-Shuaiybah (SB), Al-Umrah (UM), Al-Zima (ZM), Asfan (AS), Bahra (BH), Central Area (CA), Madrakah (MD), and South Makkah (SM). The study area is approximately 11 801 km^2, while the population included approximately 2 612 102 people as of 2022[24].

Makkah has an arid climate with a tropical temperature range, like most cities in the Arabian Peninsula. Due to Makkah's location in the tropical region and its relative distance from the Red Sea coast, it has a dry climate, with very high temperatures in the summer, reaching approximately 47 ℃ in June. In the winter season, Makkah differs from the rest of the cities on the Arabian Peninsula, as it is characterized by a warm climate, and temperature ranges between 25 ℃ in the morning to 17 ℃ in the evening.

2.2. Methods

Mosquito surveys were conducted in 16 municipalities of Makkah, which were divided depending on local administrative precincts (Holy Makkah Municipality). Explorations for potential oviposition sites were carried out by 120 workers. These workers were divided into 24 teams; each team contained one team leader, two survey personnel, and two assistants. Two teams served each municipality, except for the small municipalities, such as MS and CA, which had one team. In addition, there were two teams for all four municipalities: AS, MD, SB, and ZM. All teams were trained 2 weeks before the study with two workshops on the theories and practical aspects of the survey method to identify the aquatic stages of mosquitoes and sample oviposition sites. WHO guidelines were used as a model and reference for the fieldwork in terms of collection, transportation, and storage[25].

2.3. Data collection (samples)

The longitudinal monitoring surveys of mosquito oviposition sites in 16 municipalities were performed between November 2022 and October 2023. These surveys were conducted during working weekdays (6 days per week). Each municipality was equipped with a car and all necessary equipment to collect mosquito aquatic stages.

The selected oviposition sites for larvae were variable, ranging from permanent ones to occasional water collections, which included seepage, irrigation canals, surface water collections, drainage water, and stagnant water. During each survey, a habitat was first visually inspected for the presence of mosquito larvae, and then, depending on the size of the larval habitat, three to five samples were taken with a soup ladle (350 mL capacity) for the large habitats and by a usual dropper for the small habitats from each larval habitat. All samples were transported to the laboratory in a thermos box, and the plastic bags were floated in relatively cold water (10 ℃-20 ℃). Larval and pupal stages were preserved in labeled specimens containing 70% ethyl alcohol.

Mosquitoes were identified using different taxonomic keys for the region by Mattingly and Knight[26], Harbac[27], and Alahmed *et al.*[28]. The number of mosquitoes (larvae and pupae) obtained from the different sites was recorded daily. A total of 120 647 larvae and 21 484 pupae were collected during the survey.

The term "indoor" has been applied to any larval habitat located within the house wall and those houses for which a survey required contacting the owner and obtaining permission to enter the property (including those on the interior side of a home fence/house yard or within the building). Any habitat outside the boundaries of houses and around the surrounding area have been identified as "outdoor" larval habitats, even if they are adjacent to the exterior wall of the house (which does not require the permission of the property owner for the survey and includes buildings under construction).

2.4. Larval habitat and climatic data

The characteristics of larval habitats used by all reported species as oviposition sites were determined and 26 types of larval habitats were identified: Animal watering (AN), Aquarium (AQ), Automotive tires (AT), Barrel (BR), Bucket (BT), Cement basin (CB), Cooler (CO), Conditioner trough (CT), Concrete water tank (CW), Drainage (DR), Fountain (FO), Household pots (HP), Irrigation (IR), Manhole (MN), Pond (PO), Plant pots (PP), Plastic water-tank (PT), Pigeons watering (PW), Sewers (SE), Swimming pool (SP), Stream (ST), Swamp (SW), Water collection-Basement (WB), Water collection-Elevator (WE), Water leakage (WL), and

Water collection-Rains (WR).

Seasonal house, container, and breteau indices (HI, CI, and BI) for *Aedes aegypti*, which are commonly used in larval surveys related to houses and containers, were used to determine larval abundance. All larval indices were related to the month and season of collection. HI was calculated as the total number of infested houses divided by the total number of houses surveyed ×100. While CI was calculated as the total number of containers found with larvae divided by the total number of containers surveyed ×100. BI was also calculated based on the use of HI and CI data, and indicates the number of positive containers per 100 houses surveyed[10].

Monthly meteorological data from November 2022 to October 2023 were obtained from the National Center of Meteorology in KSA. Data on ambient temperature and relative humidity were given as maximum, minimum, and mean values and expressed in degrees Celsius (℃) and percentage (%), respectively. Total rainfall, wind speed, and monthly sunshine data were supplied as mm, km/h, and hours, respectively, as shown in Supplementary Table 1. In addition, the characteristics of larval habitats that were recorded and used for the analysis of the most predominant three species in the larval sampling habitats included water temperature (℃), pH, total dissolved salts (TDS) (ppm), and the degree of water turbidity (clear or turbid). Furthermore, vegetation, algae, and shade were either present or absent. These physical and chemical factors were recorded for all randomly reported mosquito larval habitats and were applied to 798 larval habitats, except irrigation (IR), which represents more than 10% of the total.

Furthermore, the occurrence frequency of oviposition sites (%) is related to the number of positive oviposition sites of the mosquito species. The joint existence among the 13 reported species was recorded to determine the most abundant/rare occurrence frequency of oviposition sites.

2.5. A field experiment design

To solve exposed PW, specially covered or tightly sealed water containers to prevent egg-laying by mosquitoes and at the same time does not hinder for pigeon drinking by using bird nipple drink waterers (called automatic bird water with nozzles).

Two models of pigeon nipple drink waterers (automatic bird water with nozzles) were designed: the first model is a rectangular plastic bucket with dimensions (40 cm length×20 cm width×20 cm height), while the second one is a circular plastic bucket with a diameter of 20 cm and a depth of 50 cm. In addition, for half-inch pipes for installation purposes, the nozzle allows water to flow when it encounters a pigeon's beak (nozzle: length 35 mm×width 10 mm).

2.6. Data analysis

Data were collected, tabulated, and analyzed using Microsoft® 365 Excel® for Windows; Release 18.2311.10710; Microsoft Corporation: Redmond, WA, USA, 2024. All statistical analyses and the correlations were performed using SPSS Statistics (SPSS for Windows; Release 29.0.2.0; IBM SPSS Statistics: Chicago, IL, USA). The meteorological data (temperature, humidity, rainfall, wind speed, and sunshine hours) during the study period were obtained but not normally distributed. Continuous variables with non-normal distributions were expressed as median (interquartile range, IQR: 25%-75%), while continuously normally distributed variables were expressed as mean±standard deviation. The *Chi-*square (χ^2) test was utilized to determine whether there was any significant difference in the occurrence of mosquito larvae and every physical factor (presence/absence) of the larval habitats. The Spearman correlation coefficient (r) was used to study the effects of water temperature, pH, and TDS on the abundance of different mosquito larvae. The means of two continuous, normally distributed variables were compared using independent samples Student's t-test, while the Mann-Whitney U test was employed to compare two groups of variables that were not normally distributed. In all statistical analyses, a P-value<0.05 was taken to express statistical significance.

2.7. Ethical approval

No ethical approval from the local government is required. This study is part of the dengue control program of Safar Company in collaboration with the Holy Makkah Municipality. The company supporting this project also bears full ethical responsibility for this research.

3. Results

3.1. Species list

Sixteen species that belong to five genera: *Aedes* (3 spp.), *Anopheles* (3 spp.), *Culex* (8 spp.), *Culiseta* (1 sp.) and *Lutzia* (1 sp.) were collected from Makkah Al-Mukarramah within the 16 municipalities during the period from November 2022 to October 2023, as shown in Table 1.

3.2. Prevalence of mosquitoes

A total of 185 608 potential mosquito oviposition sites were surveyed; of these, 154 726 (83.4%) were water sources for mosquito larvae. A total of 7 663 (5.0%) of these sources were positive for larvae. The data are shown at the genera and species levels in Table 1. A total of 142 131 mosquito larvae (L=120 647) and pupae (P=21 484) were collected. In addition, the number of positive larval habitats as well as the numbers of immature stages (larvae and pupae) were recorded throughout the municipalities as shown in Figure 2A.

3.3. Larval habitat

3.3.1. Types of mosquito larval habitats

26 Types of larval habitats were identified in several locations. In this survey, out of the 7 663 positive habitats, 3 516 (45.9%) were indoors (ID), while 4 147 (54.1%) were outdoors (OD). In addition, the majority of positive larval habitats were recorded in SQ [1 093 (545 ID, 548 OD)], followed by SR [1 003 (395 ID, 608 OD)], UM [984 (438 ID, 546 OD)], AZ [740 (341 ID, 399 OD)], and then the other municipalities [3 843 (1 797 ID, 2 046 OD)], as shown in Figure 2B.

3.3.2. Physicochemical parameters of larval habitats

The physicochemical factors were performed on all randomly reported larval habitats for the three dominant mosquitoes as shown in Table 2. By excluding irrigation (IR) due to its low data, mean±SD were calculated for all collected data, where the water temperature was the highest in CW (29.9±3.7) ℃ and lowest in SW (21.3±3.6) ℃. TDS was the highest in both WE [891.0 (477.5, 1 712.5)] and WR [160.0 (140, 195)] ppm and the lowest in CT [202.0 (131.5, 254.0)] ppm. Moreover, the Mann Whitney *U* test, which is based on median was the highest in ST (950%) and the lowest SE (41%). The pH was weak basic in all larval habitats, ranging from (7.8±0.6) to (9.0±0.4) in SP and MN, respectively.

Table 1. List of mosquito species collected from Makkah Al-Mukarramah, including positive larval habitats and number of immature stages.

No.	Full name species	No. of oviposition sites (%)						No. of immature stages (%)		
		Total	Months					Larvae	Pupae	Total
			Nov.	Dec.	Jan.	Feb.	Mar.			
1	*Aedes* (*Fredwardsius*) *vittatus* (Bigot, 1861)	205	44	22	95	9	2	1 708	649	2 357
2	*Aedes* (*Ochlerotatus*) *caspius* (Pallas, 1771)	3	0	1	0	0	0	17	0	17
3	*Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762)	5 561	913	603	1 019	531	567	76 680	16 799	93 479
	Total of *Aedes*	5 769	957	626	1 114	540	569	78 405	17 448	95 853
4	*Anopheles* (*Cellia*) *dthali* Patton, 1905	49	7	8	5	2	2	311	34	345
5	*Anopheles* (*Cellia*) *sergentii* (Theobald, 1907)	39	12	1	2	0	0	337	24	361
6	*Anopheles* (*Cellia*) *stephensi* Liston, 1901	4	2	1	0	0	0	7	5	12
	Total of *Anopheles*	92	21	10	7	2	2	655	63	718
7	*Culex* (*Barraudius*) *pusillus* Macquart, 1850	1	0	0	1	0	0	2	0	2
8	*Culex* (*Culex*) *laticinctus* Edwards, 1913	10	0	1	0	2	0	245	9	254
9	*Culex* (*Culex*) *mattinglyi* Knight, 1953	1	0	0	0	1	0	7	0	7
10	*Culex* (*Culex*) *pipiens* Linnaeus, 1758	967	181	119	201	42	93	21 018	1 609	22 627
11	*Culex* (*Culex*) *quinquefasciatus* Say, 1823	704	63	79	318	74	36	17 898	2 143	20 041
12	*Culex* (*Culex*) *sitiens* Wiedemann, 1828	5	1	0	0	4	0	90	1	91
13	*Culex* (*Culex*) *tritaeniorhynchus* Giles, 1901	94	19	14	12	4	1	2163	180	2 343
14	*Culex* (*Culex*) *univittatus* Theobald, 1901	10	0	0	2	7	0	137	20	157
	Total of *Culex*	1 792	264	213	534	134	130	41 560	3 962	45 522
15	*Culiseta* (*Allotheobaldia*) *longiareolata* (Macquart, 1838)	2	0	0	1	0	0	3	6	9
16	*Lutzia* (*Metalutzia*) *tigripes* (de Grandpre & de Charmoy, 1901)	8	0	0	2	3	2	24	5	29
	Total	7 663	1 242	849	1 658	679	703	120 647	21 484	142 131

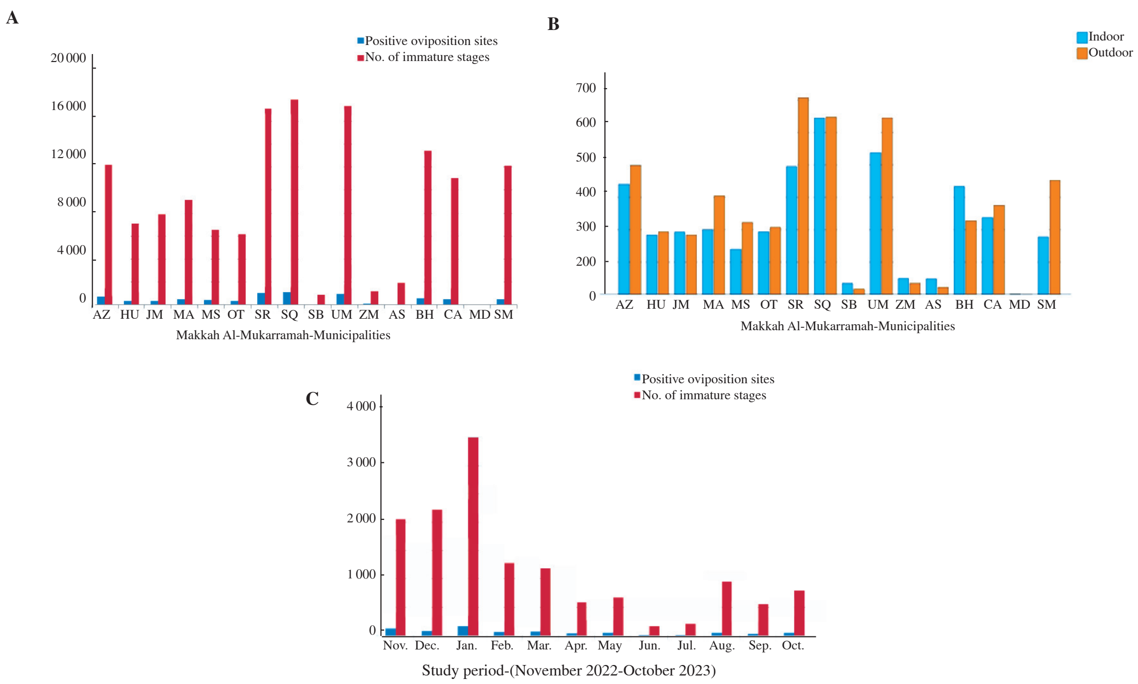

Figure 2. Positive larval habitats and number of immature stages. A. Throughout the municipalities, B. Throughout the study period, C. Throughout the municipalities (Outdoor/Indoor). Abbreviation: AZ, Al-Aziziya, HU, Al-Hudaybiyah, JM, Al-Jumum, MA, Al-Ma'abdah, MS, Al-Masfalah, OT, Al-Otaybiya, SR, Al-Sharayia, SQ, Al-Shawqiya, SB, Al-Shuaiybah, UM, Al-Umrah, ZM, Al-Zima, AS, Asfan, BH, Bahra, CA, Central Area, MD, Madrakah, SM, South Makkah.

3.3.3. Frequency of positive larval habitats

All types of larval habitats were found to have the reported immature stages of mosquitos, but their prevalence differed from one source to another and was different depending on the season. Of the 7 663 positive habitats, the immature stages of *Ae. aegypti* were found the highest number of times (frequencies) (5 561, 72.5%) among all types of larval habitats, followed by *Cx. pipiens* (967, 12.6%), and then *Cx. quinquefasciatus* (704, 9.2%).

The most common water sources that were positive for all mosquito larvae were PW (3 043, 39.7%), BT (1 275, 16.6%), WL (479, 6.3%), and AT (436, 5.7%), as shown in Supplementary Figure 1. In addition, the most common water sources that were positive for *Ae. aegypti* larvae were PW (2 387, 42.9%), BT (1 000, 18%), WL (341, 6.1%), HP (318, 5.7%), AT (279, 5%), PP (267, 4.8%), CW (229, 4.1%), CO (224, 4%), BR (121, 2.2%), WR (90, 1.6%), WB (68, 1.2%), and then the other types of larval habitats (237, 4.3%).

3.4. The extent of the Aedes aegypti population

To determine and assess the temporal variations of *Ae. aegypti* larval abundance, HI, CI, and BI were used to calculate larval indices, which were related to month and season of collection. These indices showed high-level infestations of artificial water containers, which included AN, AQ, AT, BR, BT, CB, CO, CW, CT, FO, HP, PW, PP, PT, and SP. In addition, the values of CI and BI did not remain static but fluctuated over time, as shown in Table 3.

3.5. Seasonal variation and fluctuation over time

3.5.1. Number of oviposition sites

A total of 7 663 residences positive for mosquito larvae exhibited seasonal variation and fluctuation over time. The majority number of positive residences for all mosquito larvae was observed in January 2023 (1 658, 21.6%), as shown in Figure 2C.

The positive residences was observed for different genera throughout the year, with peaks during November 2022 to March

Table 2. The physiochemical parameters of different larval habitats in study area.

Habitat type	*n*	pH	Water temperature (℃)	TDS (ppm)^
Animal watering (AN)	3	8.6±0.5	27.1±0.5	130 (130, 150)
Aquarium (AQ)	2	7.9±0.5	25.5±0.1	183 (129.750, 144.750)
Automotive tires (AT)	18	8.5±0.4	26.5±4.2	161 (75, 250)
Barrel (BR)	43	8.5±0.5	23.6±3.7	295 (146, 578)
Bucket (BT)	36	8.3±0.3	24.0±3.7	191 (140, 630)
Cement basin (CB)	30	8.7±0.4	24.8±2.9	330 (173.5, 842.5)
Cooler (CO)	80	8.4±0.5	27.2±5.9	199 (135, 254)
Conditioner trough (CT)	53	8.6±0.4	22.1±0.4	202 (131.5, 254.0)
Concrete water-tank (CW)	80	8.6±0.5	29.9±3.7	587.50 (200.25, 957.50)
Drainage (DR)	22	8.5±0.3	23.8±4.7	195.5 (146.5, 302.5)
Fountain (FO)	10	8.3±0.3	27.5±2.6	145 (130.00, 414.75)
Household pots (HP)	30	8.6±0.5	22.6±3.9	165 (134.0, 296.5)
Irrigation (IR)	1		na	
Manhole (MN)	11	9.0±0.4	24.4±6.1	574 (481.5, 1 112.5)
Pond (PO)	25	8.7±0.5	27.7±2.6	601 (433.5, 930.0)
Plant pots (PP)	39	8.6±0.6	23.6±3.9	365 (274, 601)
Plastic water-tank (PT)	24	8.3±0.5	25.5±5.3	45 (264.25, 705.75)
Pigeons watering (PW)	234	8.4±0.4	23.3±4.8	155.50 (134.75, 220.0)
Sewers (SE)	55	8.8±0.4	26.3±5.8	41 (362, 771)
Swimming pool (SP)	13	7.8±0.6	25.2±3.7	885.50 (483.50, 1 350.25)
Stream (ST)	3	8.3±0.5	22.1±5.5	950 (510, 1 560)
Swamp (SW)	8	8.7±0.5	21.3±3.6	885.50 (483.50, 1350.25)
Water collection-basement (WB)	20	8.5±0.4	25.2±3.2	714 (606.25, 901.50)
Water collection-elevator (WE)	12	8.4±0.3	24.4±3.5	891 (477.50 1 712.5)
Water leakage (WL)	16	8.4±0.4	28.6±4.6	156.50 (140.0, 187.5)
Water collection-rains (WR)	12	8.4±0.3	24.4±3.5	160 (140, 195)

^Data expressed as median (Q1, Q3). na: not available. TDS: total dissolved salts.

Table 3. The relative measurements of *Aedes aegypti* population over time.

Months	House index (HI)			Container index (CI)			Breteau index (BI)		
	Number of inspected houses	Number of infested houses	HI (%)	Number of inspected containers	Number of positive containers	CI (%)	Number of inspected houses	Number of positive containers	BI (%)
Nov. 2022	8 979	229	2.6	9 954	844	8.5	8 979	844	9.4
Dec. 2022	7 659	288	3.8	8 297	522	6.3	7 659	522	6.8
Jan. 2023	9 314	532	5.7	10 231	885	8.7	9 314	885	9.5
Feb. 2023	5 949	295	5.0	7 817	483	6.2	5 949	483	8.1
Mar. 2023	6 046	318	5.3	7 067	517	7.3	6 046	517	8.6
Apr. 2023	6 229	144	2.3	6 987	262	3.7	6 229	262	4.2
May 2023	7 652	213	2.8	8 229	350	4.3	7 652	350	4.6
Jun. 2023	6 528	76	1.2	6 917	108	1.6	6 528	108	1.7
Jul. 2023	5 972	48	0.8	6 791	98	1.4	5 972	98	1.6
Aug. 2023	6 951	139	2.0	8 211	343	4.2	6 951	343	4.9
Sept. 2023	7 295	85	1.2	8 624	217	2.5	7 295	217	3.0
Oct. 2023	7 369	115	1.6	7 989	339	4.2	7 369	339	4.6
Total/Average	85 943	2 482	2.9	97 114	4 968	5.1	85 943	4 968	5.8

2023 (from late autumn until early spring) for *Aedes* larvae (3 806 oviposition sites, 66.0% of total *Aedes* spp.), and *Culex* larvae (1 275 oviposition sites, 71.1% of total *Culex* spp.). The majority numbers of both genera were observed in January 2023 (1 114, 19.3%) and (534, 29.8%), respectively.

The most positive residences for mosquito larvae recorded were *Ae. aegypti* (5 561 oviposition sites), where the majority number was observed in January 2023 (1 019, 18.3%), November 2022 (913, 16.4%), December 2022 (603, 10.8%), and then in both March (567, 10.2%) and February 2023 (531, 9.5%), as shown in Table 1. In addition, the second total of positive residences was recorded for *Cx. pipiens* larvae (967), where the majority number was observed in January 2023 (201, 20.7%), November (181, 18.7%), and December 2022 (119, 12.3%) and then March 2023 (93, 9.6%). Moreover, the third total was recorded for *Cx. quinquefasciatus* larvae (704), where the majority number was observed in January 2023 (318, 45.1%) and then in December 2022 (79, 11.2%) and February 2023 (74, 10.5%). Furthermore, the fourth and fifth totals of positive residences were recorded for larvae of *Ae. vittatus* (205) and *Cx. tritaeniorhynchus* (94), respectively. The majority numbers of *Ae. vittatus* and *Cx. tritaeniorhynchus* were observed in January 2023 (95, 46.3%) and November 2022 (19, 20.2%), respectively.

3.5.2. Number of immature stages (larvae and pupae)

The total number (142 131) of mosquito larvae and pupae showed significant variations between months. The majority number of all immature stages was collected in January 2023 (34 967, 24.6%), as shown in Figure 2C.

The largest number of immature stages of mosquito genera was collected throughout the year, with peaks from November 2022 to March 2023 for *Aedes* larvae and pupae (68 376, 71.3% of total *Aedes* spp.) and then for *Culex* larvae and pupae (33 675, 74.0% of total *Culex* spp.). The majority of both genera were collected in January 2023 (22 537, 23.5%) and (12 389, 27.2%), respectively.

At the level of species, the largest population size of mosquito larvae and pupae was collected for *Ae. aegypti* (93 479), the population size started high in November 2022 (13 396, 14.3%) and remained static to the next December (13 946, 14.9%) until it attained a peak in January 2023 (21 432, 22.9%). The size gradually decreased thereafter from February (8 955, 9.6%) to May (4 907, 5.2%) and then decreased substantially in June (1 223, 1.3%) and July (1 480, 1.6%), before rising again from August (6 613, 7.1%) onwards. In addition, the second largest population size of larvae and pupae by species was *Cx. pipiens* (22 627), where the majority number was collected in January 2023 (4 375, 19.3%), December (4 149, 18.3%), November 2022 (3 715, 16.4%) and then March 2023 (2 315, 10.2%). The third total number was for *Cx. quinquefasciatus* (20 041), where the majority number was collected in January 2023 (7 656, 38.2%), December 2022 (2 970, 14.8%), February 2023 (2 412, 12.0%), and then November 2022 (2 266, 11.3%) The fourth and fifth total numbers of larvae and pupae were for *Ae. vittatus* (2 357) and *Cx. tritaeniorhynchus* (2 343), respectively. The majority numbers of *Ae. vittatus* and *Cx. tritaeniorhynchus* were observed in January 2023 (1 105, 46.9%) and December 2022 (816, 34.8%), respectively.

3.6. Correlation between habitat characteristics and larval occurrence

The study of physical-chemical properties of water in the oviposition sites revealed that TDS varied between 30 and 2 750 ppm, pH ranged between 7.1 and 10.7, and water temperature varied between 12.8 ℃ during winter and 36 ℃ during summer. The Spearman correlation coefficient results indicated that, there was a significant negatively correlation between two larval species (*Ae. aegypti* and *Cx. pipiens*) with water temperature and TDS (r=-0.199, P=0.001) and (r=-0.220, P<0.001) for the former, and (r=-0.191, P=0.002) and (r=-0.213, P<0.001) for the latter. Also, there was a negatively associated between two larval species of (*Cx. pipiens* and *Cx. quinquefasciatus*) with pH (r=-0.132, P=0.030) and TDS (r=-0.151, P=0.016), respectively.

In addition, analysis using the *Chi*-square test (χ^2) indicated that *Ae. aegypti* larvae were more likely to be present in habitats with clear water (P<0.01), without vegetation (P<0.01), and in shaded habitats (P<0.01). *Cx. pipiens* larvae were significantly associated with habitats without vegetation (P<0.01), with algae (P<0.01), and with shaded habitats (P<0.01). *Cx. quinquefasciatus* larvae were more likely to be present in habitats without vegetation (P<0.01), with algae (P<0.01), and occupy shaded habitats (P<0.01), as shown in Table 4.

3.7. Associated species

A total of 41 different forms (306 associated oviposition sites) of associations were reported. Of these associations, two forms of *Ae. aegypti* with *Cx. pipiens* and *Cx. quinquefasciatus* were common (259 oviposition sites, 84.6%). The most potentially associated species was *Ae. aegypti* with nine other species, particularly *Cx.*

Table 4. Mosquito larval occurrence association with some of larval habitat characteristics.

Habitat characteristics		*Aedes aegypti*			*Culex pipiens*			*Culex quinquefasciatus*		
		n (%)	χ^2	*P*-value	*n* (%)	χ^2	*P*-value	*n* (%)	χ^2	*P*-value
Turbidity	Turbid	892 (15.7)	2 689	<0.001	2 155 (48.4)	4.85	0.28	1 815 (50.1)	0.028	0.87
	Clear	4 807 (84.3)			2 302 (51.6)			1 805 (49.9)		
Vegetation	Presence	245 (4.3)	4761	<0.001	833 (18.7)	1 747.7	<0.001	881 (24.3)	953.6	<0.001
	Absent	5 454 (95.7)			3 624 (81.3)			2 739 (75.7)		
Algae	Present	2 819 (49.5)	0.65	0.42	2 507 (56.2)	69.6	<0.001	2 059 (56.9)	68.5	<0.001
	Absent	2 880 (50.5)			1 950 (43.8)			1 561 (43.1)		
Shade	Shaded	3 518 (61.7)	313	<0.001	2 589 (58.1)	116.6	<0.001	2 126 (58.7)	110	<0.001
	Unshaded	2 181 (38.3)			1 868 (41.9)			1 494 (41.3)		

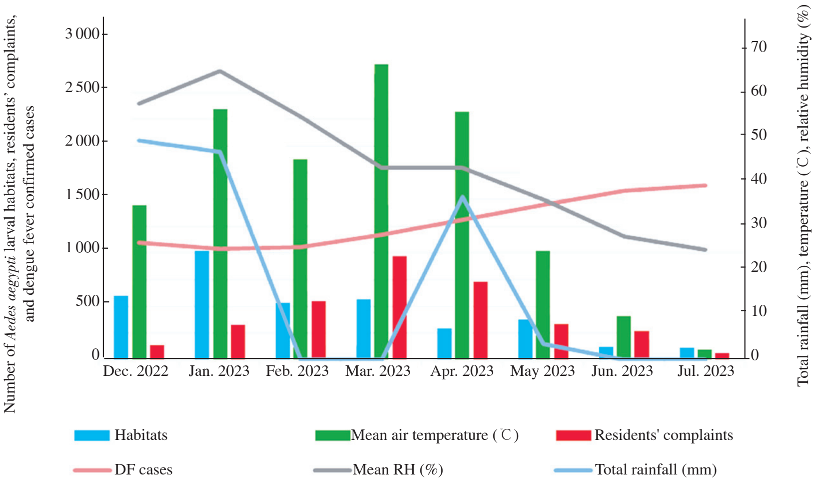

Figure 3. The association among the monthly number of positive residences for *Aedes aegypti* larvae, dengue fever confirmed cases, number of residents' complaints, and the environmental factors (rain-fall, air temperature, relative humidity). RH: Relative humidity.

pipiens (in 140 oviposition sites, 45.7%) and *Cx. quinquefasciatus* (119 oviposition sites, 38.9%). The second most associated species was *Cx. pipiens* with seven other species. Following this, both *Cx. quinquefasciatus* and *Anopheles dthali* were associated with five species separately, and *Ae. vittatus* was associated with four species, particularly *Cx. quinquefasciatus* (12 oviposition sites, 3.9%) and *Ae. aegypti* (9 oviposition sites, 2.9%). The association between *Cx. pipiens* and *Cx. quinquefasciatus* was rare (together in one oviposition site). In addition, the association of the rest of the species was rare and ranged from one to four oviposition sites, according to Supplementary Table 2.

3.8. Realistic case study (Aedes aegypti)

A realistic case study of *Ae. aegypti* was carried out to understand the growth pattern of the mosquito vector, considering its larval stages, and to reveal the evident frequent factors associated with the spread of dengue fever. The realistic case study looked for correlations among numbers of positive larval habitats, residents' complaints, confirmed cases of dengue fever, and environmental factors and was carried out during the moderate climatic period (January to June 2023) for vector spread, and then for dengue fever transmission, as shown in Figure 3.

3.8.1. Number of positive larval habitats

During the case study period, monthly variations in the number of residents positive for *Ae. aegypti* larvae were observed. The highest numbers of larval habitats and then number of larvae were observed in the duration between December 2022 and March 2023, whereas the highest number of larval habitats was recorded in January 2023 (1 019 sites). Distribution of positive larval habitats of *Ae. aegypti* as shown in Supplementary Figure 2.

3.8.2. Environmental factors (air temperature, relative humidity, and rainfall)

The correlation between larval habitats and environmental factors (temperature, relative humidity, and rainfall) mostly showed a reasonable association between them. When the mean ambient

Table 5. Correlation among number of residences positive for *Aedes aegypti* larvae with numbers of residents' complaints, dengue fever confirmed cases, and the environmental factors (December 2022-July 2023).

Items		Number of positive larval habitats	Residents' complaints	Dengue fever confirmed cases	Mean air temperature (℃)	Mean RH (%)	Total rainfall (mm)	Mean wind speed (km/h)
Number of positive larval habitats	*r*	1.000	0.462	-0.409	-0.843	0.951	0.642	-0.445
	P-value	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Dengue fever confirmed cases	*r*	-0.409	0.539	1.000	0.209	-0.449	-0.748	0.190
	P-value	<0.001	<0.001	-	<0.001	<0.001	<0.001	<0.001

r-Spearman correlation coefficient. RH: Relative humidity.

temperatures ranged from 24.2 ℃ to 27.4 ℃ between December 2022 and March 2023, the mean relative humidity ranged from 42% to 63% in the same period with a maximum in January 2023 (63%). Finally, the largest volume of precipitation was recorded in December 2022 (47.9 mm), followed by January 2023 (45.3 mm), and then April 2023 (35.6 mm).

3.8.3. The number of residents' complaints

The person reporting the complaint will contact the main complaints reception center (Baladi platform) of the Holy Makkah Municipality, which specializes in complaints about disturbances caused by mosquitoes. Residents' monthly complaints were highest in the period from December 2022 to May 2023 (reaching 1 022 to 2 765), with the highest number recorded in March 2023 (2 765 complaints), followed by January 2023 (2 346), and then April 2023 (2 320).

3.8.4. Number of dengue fever cases

The number of dengue fever incidents during the winter to early summer in 2023 (3 169 cases distributed as shown in Supplementary Figure 2) was 25 times higher compared with 2022 (127 cases). In 2023, the highest number of cases was reported in March (972), while in 2022, the highest number of cases was reported in December (141).

The number of positive residences for *Ae. aegypti* larvae showed significant variations between months. The number of positive residences exhibited a significant negative correlation with temperature, rainfall, wind speed, and dengue fever cases, in contrast with a significant positive correlation with residents' complaints and relative humidity, as shown in Table 5. In addition, the number of dengue fever cases showed significant correlation with environmental factors (temperature, relative humidity, and rainfall), as shown in Table 5.

3.9. Field experimental study

A field experiment was conducted. Three locations known to have a high density of pigeons in the study area were randomly selected. The target sites were surveyed the night before the experiment to get rid of the exposed pigeons' waterers and replace them with new models of automatic bird water with nozzles (Supplementary Figure 3). The new models have proven their effectiveness if all traditional waterers are removed in advance.

4. Discussion

Knowledge of vector populations in different geographical regions is required to carry out effective vector control strategies and disease management. Our findings provide a better understanding of the prevalence and distribution of mosquito larvae and of disease transmission in Makkah Al-Mukarramah. These data increase our ability to predict transmission and to design and implement control measures accordingly to prevent outbreaks of the diseases. It is essential to link meteorological conditions with entomological surveillance data to pinpoint the time and place of potential disease outbreaks.

In this study, the mosquito fauna of Makkah included 16 taxa of mosquitoes. Of these, three species of two genera (*Aedes* and *Culex*) *Ae. aegypti*, *Cx. pipiens*, and *Cx. quinquefasciatus* were the most predominant species and the most abundant mosquitoes encountered, which poses a threat to public health. The fourth and fifth most common species encountered are the most important as arboviral vectors, *Ae. vittatus* and *Cx. tritaeniorhynchus*, which are vectors of yellow fever (in several African countries)[29], and Rift

Valley fever and Japanese encephalitis[30,31], respectively. Thus, the abundance and vectorial roles of the most common species reported in this study require attention and regular monitoring.

Makkah has a unique geographical and topological location. The main Al-Hijaz mountains pass through the middle of the province, creating two main sectors: a mountainous area known as the "Al-Sarawat Mountain range", and a lowland coastal plain on the western side along the coast of the Red Sea known as "Tihama"[32].

In Tihama, the weather conditions promote dense vegetation cover. In the Al-Sarawat Mountain range, the weather is cooler due to the high altitude. In addition, the tops of the mountains face the clouds, forming a "fog oasis", which creates a unique ecosystem. Therefore, the region is rich in flora and characterized by its great species diversity and denseness[33].

The flora is a mixture of Asian, African and Mediterranean Irano-Turanian plant species[34]. There are148 species belonging to 125 genera and 44 families are known along the escarpment of Makkah[33], forming an enormous variety of habitats for insect species including mosquitoes.

The energy for all life-sustaining activities of both female and male mosquitoes is provided by plant sugars, usually nectar from flowers. In addition, native plants, as well as invasive alien plant species, play a role in mosquitoes' survival and vectorial capacity[8,35]. In Makkah, most flowering plant species are seen in meadows, mountains, and wadis (valleys), which are dominated by several species, as shown in Supplementary Table 3[8,33,34].

Finally, *Prosopis juliflora* D.C. is a shrub in the family Fabaceae. The plant is one of the worst invasive alien plants in many parts of the world; it occupies millions of hectares in several countries, including KSA. This plant acts as a sugar source for the survival of mosquitoes, especially during dry periods when sugar sources from native plants are largely unavailable. In addition, invasive plants are actively grow and flower for long periods and could significantly contribute to mosquito longevity and thereby enhance disease transmission potential.

The combination of meteorological factors with the physiographical structure of the region, as well as the behavior of mosquitoes that venture into homes for shade due to the hot season, has led to a vector population that is larger and that can tolerate the dry season and thus trigger disease outbreaks, such as dengue fever. These findings call for further investigations of the factors enabling *Ae. aegypti* to adapt to the arid climatic conditions in Makkah[11].

To determine the larval abundance of *Ae. aegypti*, HI, CI, and BI were calculated to diagnose dengue fever incidence risks. These indices showed high-level infestations of 15 artificial water containers. The main artificial water containers that were positive for *Ae. aegypti* larvae were PW (2 387, 42.9%), followed by BT (1 000, 18%).

According to the WHO, an area is at high risk of dengue fever incidence when these indices are above a threshold of 5% for HI and BI and 3% for CI. In the present study throughout the year, HI ranged from 0.8%-5.7% with an average of 2.9%, CI ranged from 1.4%-8.7% with an average of 5.1%, and BI ranged from 1.6%-9.5% with an average 5.8%. From the larval abundance data obtained, it is clear that Makkah is an area with a high level of dengue fever incidence and is within the range to require attention, as the container-developing mosquitoes are adequate to cause outbreaks.

Water temperature, pH, and TDS of the developing larval water were measured to examine the effect of such factors on larval occurrence. Most of the larval habitats in this survey that were appropriate for mosquito larvae contained algae, which has also been found in other studies[6]; in addition, mosquito larvae prefer shaded habitats, which has also been found previously[10,11]. The species distribution of mosquito larvae varied based on the studied habitat types. For example, *Ae. aegypti* was found in all reported larval habitats except for MN & SW, and *Cx. pipiens*, and *Cx. quinquefasciatus* were found in all reported larval habitats except for IR & ST, and for AN, AQ, CT & IR, respectively. The correlation revealed that the unvegetated and shaded habitats were more likely to be occupied by the three species, especially for *Ae. aegypti*, which preferred small and shaded habitats to lay their eggs, whereas the presence of algae was the most preferable habitat for *Cx. pipiens*, and *Cx. quinquefasciatus*. The result also showed no significant correlation between the pH of the water and the distribution of different mosquito larvae of *Ae. aegypti* and *Cx. quinquefasciatus* as mentioned by Alahmed *et al.*[36].

The current study demonstrated that the three mosquitoes, *Ae. aegypti*, *Cx. pipiens*, and *Cx. quinquefasciatus* were the most predominant associated species within the larval sampling habitats. The former is the main vector of dengue fever, while the latter two are known to be the most important vectors of West Nile virus, the incidence of which is increasing in many parts of the world, including the Arabian Peninsula. This flavivirus is maintained through the avian and mosquito transmission cycle in nature[37]. In addition, many people raise birds indoors[10,12]. Thus, attention should be given to the joint existence and occurrence frequency of oviposition sites for control of the main vectors, especially since the nature of the area and people's practices of poultry farming and water storage contributes to mosquito productivity.

In dengue fever endemic regions, the dynamics of dengue vectors and dengue occurrence are seasonal[38]. This was also evident in our case study by the peak in the number of dengue fever cases in March 2023 (972), and by the frequent changes in the size of the vector mosquito population, with a peak in positive larval habitats of *Ae. aegypti* in January 2023 (1 019). This was immediately followed by a successive increase in confirmed dengue fever cases in March. At the same time, Makkah also received the largest number of arrivals from abroad to perform the Ramadan Umrah rituals during this period (March/April), and the number of pilgrims reached 1 174 551 people[39], which increases the possibility of the emergence of the virus among people arriving from endemic countries. During the outbreak period, the maximum number of confirmed cases, residents' complaints, and positive larval habitats were recorded between the two times it rained in January to April 2023; a peak of cases occurred in early spring in the first quarter of the year. In addition, April was the turning point in the disease outbreak and the increase in residents' complaints when all the appropriate climatic conditions for the outbreak and the increase came together.

The number of positive residences for *Ae. aegypti* larvae exhibited a significant negative correlation with dengue fever cases, in contrast with a significant positive correlation with residents' complaints and a high significant positive correlation among the dengue fever cases and residents' complaints, as shown in Supplementary Figure 4. Dengue fever cases also revealed a significant positive correlation with temperature, in contrast to a significant negative correlation with relative humidity and rainfall. The study concluded that factors such as temperature and rainfall are the most contributing factors to the spread of disease vectors and, thus, dengue. Post-rainfall creates favorable breeding environments for dengue mosquito vectors, increasing transmission rates. Summer heat also accelerates the mosquito life cycle and enhances viral replication, increasing the risk of dengue transmission[23,40].

This study will help in developing appropriate control strategies to reduce the risks of disease transmission and spread in KSA because of human practices. Our study will change the unhealthy behaviors of some people, which were watering and feeding pigeons with leftover food from rice, bread, and oily foods, for example, which causes many health and visual problems, especially on sidewalks, walking places, and next to homes. Of note, our findings show that more than 40% of positive residences for *Ae. aegypti* larvae were PW. Moreover, *Ae. aegypti* has adapted to the arid climatic conditions in Makkah because of *Prosopis julifora*, which attracts mosquitos and is used as a source of sugar meals. Among these strategies is the development of innovative integrated vector management, such as reducing mosquito populations as well as reducing the vectorial capacity and longevity of mosquitoes. Therefore, we propose new appropriate alternatives to mechanical, biological, and environmental control in this current study.

The highest proportion of oviposition sites for *Ae. aegypti* larvae were found in PW (42.9%). To solve exposed PW, specially covered or tightly sealed water containers for pigeon drinking should be used or bird nipple drink waterers (automatic bird water with nozzles), as shown in Supplementary Figure 3.

Previous news articles have proven that watering pigeons is one of the most important habitats that the *Ae. aegypti* mosquito prefers to lay its eggs in by up to 50% in Makkah Al-Mukkarramah[41]. Accordingly, the Holy Makkah Municipality has attempted during previous years to find solutions to replace the traditional and exposed pigeon waterer with a new design that was distributed in all districts[42]. The new watering design did not yield any fruitful results, and the problem continues with the increasing cases of dengue fever because there is no radical and effective solution to this problem.

This experiment urges the use of "bird nipple drink waterers" in KSA to reduce the potential positive residences for *Ae. aegypti* larvae by more than 40% as an alternative to traditional exposed pigeon watering. In addition, legislation must be issued requiring users, both citizens and residents, to use the proposed new pigeon watering and criminalizing those who violate these instructions.

Our work suggests introducing host-specific and damaging biological control agents, such as the leaf-tying moth *Evippe* sp. (Gelechiidae), as a biocontrol agent against mesquite (*Prosopis juliflora*). This may be a cost-effective and safe way of reducing the impact of invasive plants as applied in Australia[43].

Environmental manipulation is another control method. This would involve removing or cutting the flowering branches of exotic plants as a means of controlling them in Makkah, as they are highly attractive to mosquitoes and provide them with sugar that is critical to their survival during dry periods[35]. Further investigations should be undertaken to determine all the native and invasive plants in Makkah and the precise timing of their flowering to understand which of these plants is the main attractant to mosquitoes as a source of sugar.

This study has potential limitations. Cultural and traditional customs and other biases prevent systematic exploration work in or around home yards. Therefore, they are subject to biases and confounding that may have an impact on the interpretation of our results, especially indoors.

The findings of this study will help in operational programs to control mosquito vectors and enable decision-makers of the state entities (KSA authorities) to develop appropriate control strategies to minimize the risk of disease transmission and outbreaks in KSA resulting from changing human activities or climate change.

These strategies may include monitoring the spread of insecticide resistance and the development of innovative, integrated vector management strategies such as reducing mosquito populations and vectorial capacity and longevity by applying new alternatives to mechanical, biological, and environmental control. In terms of mechanical control, this study urges the use of "bird nipple drink waterers". In terms of biocontrol, the work suggests introducing a host-specific biocontrol agent against mesquite (*Prosopis juliflora*), such as the leaf-tying moth (Gelechiidae). As an environmental control, this work proposes removing or cutting the flowering branches of exotic plants that are attractive to mosquitoes. In addition, use of new mosquito biocontrol methods that reduce pathogen transmission and overall disease burden should be considered, such as using *Wolbachia* strains to infect natural populations of *Ae. aegypti* to reduce dengue fever transmission risk. We hope to use *Wolbachia* strains in Makkah in a new field study of this approach, which has previously been applied in the neighboring city of Jeddah from 2022.

Furthermore, one of these strategies is using new environmentally friendly tools and cheap mosquito control, especially indoor, so that it is under the control of stakeholders. For example, traps have been designed for automotive tire recycling called *Ovillanta*, which attracts gravid female mosquitoes to lay eggs and then disposes of the larvae after they hatch. This study recorded that automotive tires are a potential habitats for mosquito larvae.

If mechanical, biocontrol, or community efforts to manage invasive plants or use strains of *Wolbachia* can be developed and implemented, they can provide an environmentally sustainable strategy to reduce the incidence of dengue fever or any other diseases. Improved management of biocontrol, particularly for control of invasive plants, will also provide many other benefits, including increased incomes for local communities Muller *et al.*[35]. Therefore, we call on the Holy Makkah Municipality to reduce the use of chemical pesticides in large quantities to eliminate pests including mosquitoes because of their negative and cumulative adverse effects on human, animal, and plant health, and to replace them with one of the alternative methods presented in this work. The average annual use of pesticides reached 71 794 liters and 5 742 kg per year for all pests[44].

It is evident that the control strategies of dengue fever vectors presently employed in Makkah are inadequate because of the species of mosquitoes and the prevalence of oviposition sites identified in this study. Thus, further studies on the ecology and bionomics of mosquitoes are required. Furthermore, the mathematical model of mosquito populations should be studied to understand mosquito vector considering life cycles in order to predict mosquito population dynamics.

Conflict of interest statement

The authors declare that there is no conflict of interest. The authors declare that there is no conflict of interest with Safar Company.

Patent

The new bird waterer model (Automatic bird water with nozzles, Supplementary Figure 3) has also been registered with the Saudi Authority for Intellectual Property as a patent, request number 43462888, on May 22, 2024.

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Authors' contributions

HB did the designing of the research, guidance of the research work, final editing, and writing and reviewing the manuscript.

KH performed data analysis, did the supervision of the research work, and reviewing of the manuscript. OA performed sampling, data collection, and did the supervision of the data analysis. RAS did the overall supervision and guidance of the research work and communicated with the Saudi entities included in the research. All authors read and approved the final manuscript.

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Supplementary Table 1. Meteorological factors in the survey area (2022/2023).

Months	**Air Temp (℃)**			**Relative Humidity (%)**			**Wind Speed (Km/h)**	**Total Rainfall (mm)**	**Monthly sunshine hrs.**
	(Max)	**(Min)**	**(Mean)**	**(Max)**	**(Min)**	**(Mean)**	**(Mean)**		**(Mean)**
Nov-22	34.6	23.7	28.5	91	26	58	3	56.1	264
Dec-22	31.2	20.8	25.6	98	23	56	3	47.9	248
Jan-23	29.2	19.7	24.2	97	28	63	3	45.3	260.4
Feb-23	31.1	19.2	24.6	90	20	53	3	0	245.8
Mar-23	34.1	21.6	27.4	86	8	42	3	0.001	282.1
Apr-23	37.2	24.6	30.6	95	12	42	4	35.6	282
May-23	41	27.6	33.8	94	10	35	3	3.6	303.8
Jun-23	44.3	30.6	37	60	10	27	4	0	321
Jul-23	44.4	32.2	38	67	12	24	3	0	313.1
Aug-23	43.1	30.9	36.6	93	13	38	3	10.4	297.6
Sep-23	44.1	31	36.9	84	11	39	3	2.5	282
Oct-23	40.4	27.2	33.2	96	8	45	3	62.4	300.7

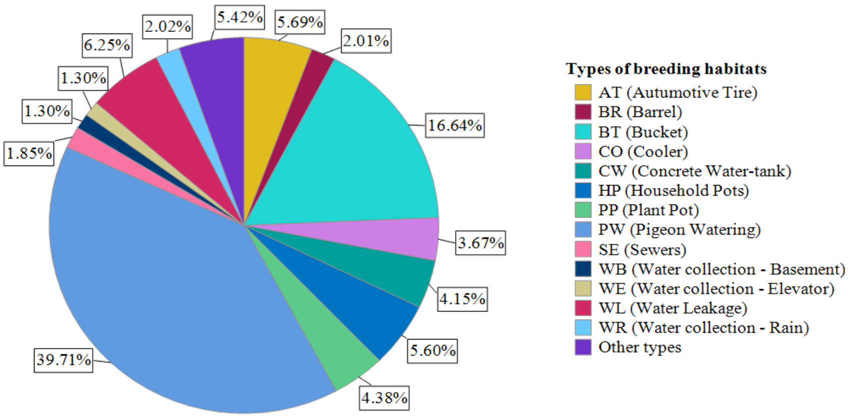
Supplementary Table 2. Different forms of association among mosquito larvae within oviposition sites in Makkah Al-Mukkaramah during the study period. (one hollow square = 1-10 breeding sites; two hollow squares = 11-50 breeding sites; one black square = > 50 breeding sites).

Species	*Ae. aegypti*	*Ae. caspius*	*Ae. vittatus*	*An. dthali*	*An. sergentii*	*An. stephensi*	*Cx. laticinctus*	*Cx. mattinglyi*	*Cx. pipiens*	*Cx. quinq.uefasciatus*	*Cx. sitiens*	*Cx. tritaeniorhynchus*	*Lt. tigripes*	**No. of oviposition sites**
Ae. aegypti			□	□	□	□		□	■	■		□	□	
Ae. caspius									□					
Ae. vittatus	□			□					□	□□				
An. dthali	□		□						□	□	□			
An. sergentii	□									□		□		
An. stephensi	□													
Cx. laticinctus									□					**306**
Cx. mattinglyi	□													
Cx. pipiens	■	□	□	□			□			□		□		
Cx. quinquefasciatus	■		□□	□	□				□					
Cx. sitiens				□										
Cx. tritaeniorhynchus	□				□				□					
Lt. tigripes	□													
Number of associated species	**9**	**1**	**4**	**5**	**3**	**1**	**1**	**1**	**7**	**5**	**1**	**3**	**1**	**41**

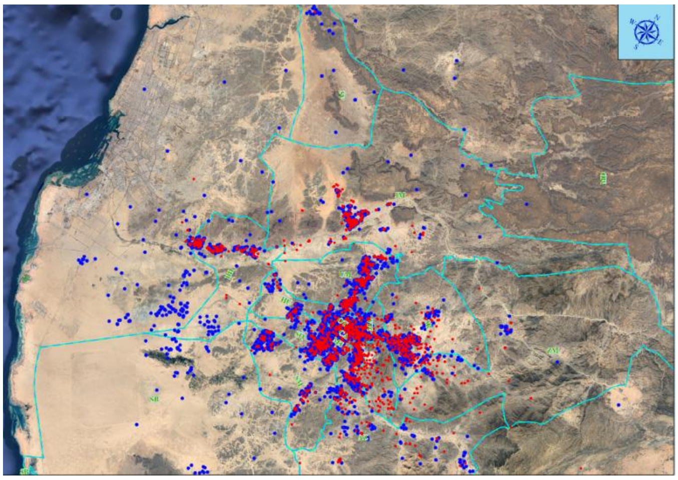
Supplementary Table 3. A list of Makkah plants that attract mosquitoes and provide them with sugar to survive.

Plant type	**Family**	**Species**
Wild	Amaranthaceae	*Aerva javanica* (Burm. f.)
		Amaranthus lividus L.
	Apocynaceae	*Calotropis procera* (Ait)
	Poaceae	*Dactyloctenium aegyptium* (L.)
		Echinochloa colonum
	Boraginaceae	*Heliotropium crispum* Desf.

	Fabaceae	*Prosopis juliflora*
Freshwater (semi-aquatic)	Poaceae	*Arundo donax* L.
	Cyperaceae	*Cyperus schimperianus* Steud.
	Poaceae	*Leptochloa fusca* (L.) Kunth
		Phragmites australis (Cav.) Steud.
	Typhaceae	*Typha domingensis* Pers.
Marshes	Aizoaceae	*Sesuvium verrucosum* Raf.

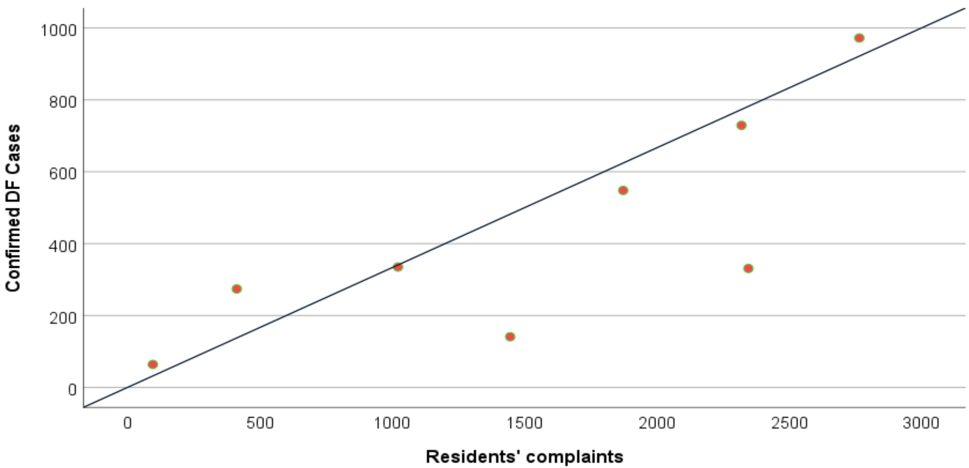


Supplementary Figure 1. Types of larval breeding habitats.



Supplementary Figure 2. Distribution of positive larval habitats of *Aedes aegypti* , and dengue fever cases during seasons of transmission.

Supplementary Figure 3. New model of a pigeon nipple drink waterer in the field.



Supplementary Figure 4. Regression relationship between confirmed dengue fever cases and residents' complaints (December 2022 – July 2023).