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Characterization and distribution of Larval Habitats of *Culex pipiens* Complex (Diptera: Culicidae) Vectors of West Nile Virus in Tabuk town, Saudi Arabia

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Abstract
The study was conducted to determine the characteristics and distribution of larval habitats of *Culex pipiens* complex in Tabuk town, Saudi Arabia. Of 116 aquatic habitats surveyed, 48 were positive for larvae of *Culex pipiens* L. (76.3%) and *Cx. quinquefasciatus* (23.7%). Habitats formed of rain-filled pools (93.8%) and leaking underground pump (6.2%) were positive for *Culex* larvae. Significantly, shallow water, mud bottom, and absence of grasses, algae, and predators were the common characteristics of habitats positive for *Culex* larvae ($P<0.05$). *Culex* larvae were also found to thrive better in stagnant habitats that devoid of solid wastes and fully exposed to sunlight. The findings of this study will help to adopt a suitable treatment of rain-filled pools and leaking underground pumps is important for larvae source management (LSM) to control the mosquito population and reduce the possible transmission of *Culex* borne-diseases in the area.

Keywords: *Culex pipiens* complex, Habitat characteristics, vector control, Tabuk, Saudi Arabia

1. Introduction

Mosquito-borne diseases threaten the lives and livelihoods of millions of people worldwide [1]. They transmit several pathogens, including protozoans, filariae, and arboviruses [2-4], they may also less frequently transmit bacterial diseases [5]. These diseases are responsible for several million deaths and hundreds of millions of cases every year. Mosquito-borne viruses are the most deadly and important diseases worldwide [1, 6]. However, West Nile Virus (WNV) is considered one of the most widely distributed mosquito-borne virus diseases in the world where it is endemic in Africana, North America, Northern Europe, and the Eastern Mediterranean regions including Saudi Arabia [7- 9]

West Nile is mainly caused by a virus that belongs to the genus *Flavivirus* and the family *Flaviviridae*. Originally, WNV was reported for the first time in Uganda in 1937 [10] and since then it has spread over wide regions including all continents except Antarctica [8]. This virus is maintained in a natural zoonotic transmission cycle between the birds and mosquitoes but it can be transmitted via mosquitoes to mammals, including humans [11]. However, the transmission of the WNV from humans to mosquitoes is uncommon, because they do not develop a high level of the virus in their blood. Therefore, humans and mammals in general are considered dead-end hosts [12, 13]. Hence, more than 80% of human infections with WNV remain asymptomatic, and, thus, many WNV cases remain undetected or unreported [14].

Mosquitoes within the genus *Culex* have been identified as the principal vectors that transmit WNV between hosts in several countries [15, 16]. Although, more than 60 mosquito species have been incriminated as a vector, *Cx. pipiens* complex has been considered as the competent vectors for WNV because this species showed a strong preference to feed on bird hosts, high abundance during the transmission season, and high infection prevalence under the field conditions [4].

WNV has been reported in many East Mediterranean countries; however, little information is available about its prevalence in equine populations in Saudi Arabia. However, infections with WNV have been detected in horses from Al-Ahsa, Riyadh, and Qateef regions [17]. However, the mosquito vector *Cx. pipiens* has been reported from several areas in Saudi Arabia [18]. This species is considered to be one of the most common and widely distributed mosquito species in the country [18].

Currently, Integrated Mosquito Management (IMM) or Integrated Vector Management (IVM) is the most recommended strategy for the control of mosquito vectors. It is known that IMM can be used as a combination of methods to control mosquitoes and hence the spread of arboviruses. Implementation of IMM for control of mosquito vectors is mainly based on an understanding of mosquito biology and the way mosquitoes spread viruses to develop plans for controlling mosquitoes. The vector control program based on the larval source management (LSM) strategy is more effective for controlling mosquito vectors. Hence, the characterization of mosquito larval habitats is a prerequisite to designing more efficient and successful mosquito control strategies. To date, few studies were conducted in Saudi Arabia to determine the preferred type of breeding habitats for *Culex* species. Therefore, the current study was carried out to characterize the larval habitats of *Cx. pipiens* complex in Tabuk town, Saudi Arabia.

2. Materials and methods

2.1. Study area

The study was conducted as cross-sectional larval surveys from January to July 2020 in Tabuk town, Saudi Arabia. Tabuk town, the capital city of the Tabuk Province is located in the northwestern part of Saudi Arabia (28°23′50″N and 36°34′44″E) (Figure 1). It is located at 773 m above the sea level and about 280 km to the Jordan-Saudi Arabia border. The area has a desert's continental weather with dry hot summer (April - October) and dry cold winter (November – March). Temperatures vary from 26 to 46 °C in the summer and from − 4 to 18 °C in winter, with widespread frosts and some not uncommon snow every few years. The rainy season in the area almost during the winter months. The precipitation ranges between 50 and 150 mm, with some not uncommon snow every 3-4 years. The total population of Tabuk city has been estimated at 455,450 people (http://worldpopulationreview.com).

2.3. Larval habitat surveys and larval collection

The study area (i.e. Tabuk town) was divided according to land use types into; 1) residential areas, 2) industrial areas, 3) valleys and 4) farmlands. In each selected area, potential aquatic habitats were examined twice a week between January and March for the presence of mosquito breeding sites. However, during April – July the larval survey was conducted once a week. All surveyed aquatic habitats for the presence of mosquito larvae were geo-referenced using GPS Magellan Triton 400, PJM Technology Industry Co., Ltd, Shenzhen Pengjin Technology Co., Ltd, Shenzhen China).

Fig 1: A map of Saudi Arabia showing the location of Tabuk are and study area (■)

Mosquito larvae were collected from possible habitats in each of the surveyed areas following the standard method. Mosquito larvae and pupae as well as aquatic invertebrates were collected from possible larval habitats using a standard 350-ml dipper and/or pipettes according to the size of the water body. In each habitat, 10 to 20 dips were made for the presence of mosquito larvae. The dipper was moved along the larval habitat, skimming the surface of the water. The dipper then lifts out of the water, mosquito larvae were counted per each dip in all surveyed habitats and poured in white trays. The water in the trays left steady until larvae and pupae raised to the surface of the water. The larvae were picked up by pasture pipettes and transferred to a plastic bottle (500 ml) contains water from the same larval habitat. The plastic bottles containing larvae were transported to the laboratory of the Department of Biology, Faculty of Science, the University of Tabuk for subsequent identification to species level.

2.4. Habitat characterization

In each selected area, the characteristics of the natural larval habitats such as types (i.e. natural/manmade), source of water, habitat status (permanent/temporary), the distance from the houses, habitat size and depth, water current, type of bottom (sand/mud), exposure to sunlight, water turbidity, temperature and pH of the water and, presence of grasses, algae and predators were recorded. The water temperature in each habitat was measured using a thermometer. The water sample (50 ml) was taken in a 500 ml plastic bottle from each of the surveyed habitats and transported to the laboratory at the Faculty of Science, University of Tabuk. In the laboratory, the pH of the water samples was measured using a pH meter (Jenway, 3500 pH meter).

2.5. Morphological identification of mosquitoes

Mosquito larvae (n = 15 larvae) were randomly selected from the field-collected immature stages from each larval habitat and examined under the dissecting microscope. The 3rd and 4th larval instars from each habitat were immediately identified morphologically to species level using proper mosquito entomological keys [19, 20]. The 1st and 2nd larval instars were left in trays provided with a small amount of larval food to develop into the 4th larval instars and then they were identified as described above.

2.6. Statistical analysis

The data obtained from this study were analyzed using a computer software SPSS ver. 22. The abundance of larval habitats and mosquito species were determined using descriptive analysis. The differences in the mean of the size, the depth, water temperature, and pH were compared between the positive and negative aquatic habitats for mosquito larvae using the T-test. The means of larval densities were compared between different land uses using analysis of variance (ANOVA) test. Moreover, associations between habitat characteristics and the occurrence of mosquito larvae were determined using the Chi-square test. The effect of water temperature and pH on the relative density of mosquito larvae in different habitats was examined using simple linear regression.

3. Results

3.1. Species composition of mosquitoes in Tabuk town

A total of 720 mosquito larvae were collected from the larval habitats. The mosquito fauna comprised one genus; *Culex* that includes two species. The two species were Cx. (*Cx.*) *pipiens* and *Cx.* (*Cx.*) *quinquefasciatus*. Of the total sampled mosquito larvae, the majority was Cx. *pipiens* (549; 76.3%).

3.2. Characteristics of mosquito larval habitats in Tabuk town, Saudi Arabia

3.2.1. Descriptive data

Table 1 shows the types of aquatic habitats, number, and percentages of positive for mosquito larvae Tabuk town. In this study, 40 field surveys were carried out to determine the occurrence of potential larval habitats. A total of 116 of aquatic bodies were surveyed for mosquito larvae, out of which 41.4% were potential larval habitats for *Culex* mosquitoes. The surveyed habitats included seven different types namely were; 1) rain-filled pools; 2) leaking underground pump; 3) water-cement tanks (for domestic use); 4) irrigated farms; 5) irrigated public gardens; 6; animal drinking water containers (half-barrels) and; 7) bird drinking water containers (i.e. small trays) (Figure 2). A higher proportion of surveyed aquatic habitats for the presence of mosquito larvae were rain-filled pools (50.0%) followed by irrigated public gardens (11.2%) and irrigated farms (10.3% for each) (Figure 3). Of these, only habitats of rain-filled pools and leaking underground pumps were positive for mosquito larvae. As shown in table 1, 77.6% (45/58) and 50% (3/6) of the surveyed rain-filled pools and water leaking underground pumps were positive for mosquito larvae, respectively. The habitats of rain-filled pools were the most common water source for mosquito breeding in the surveyed area (93.8%).

3.2.2 Association of *Culex pipiens* complex larvae with aquatic habitat characteristics

Association of the presence of *Cx. pipiens* complex with different characteristics of larval habitats is depicted in table 2. Larvae of both *Cx. pipiens* and *Cx. quinquefasciatus* were highly associated with natural ($\chi2$= 62.691; P = 0.00), temporary ($\chi2$= 8.578; P = 0.003) habitats with mud bottom ($\chi2$ = 7.725; P = 0.00). Larvae of these species were also found significantly to prefer habitats with no grasses ($\chi2$ = 39.891; P = 0.00), predators ($\chi2$ = 5.258; P = 0.022) and algae ($\chi2$ = 68.89; P = 0.00). Although there were no significant differences, all positive larval habitats were stagnant water devoid of solid wastes (i.e. clear water) and fully exposed to sunlight. Significant differences were also recorded between habitats negative and positive for *Cx. pipiens* and *Cx. quinquefasciatus* larvae in water depth (P = 0.002), pH (P = 0.01) and water temperature (P = 0.00) (Table 3).

The results of linear regression analysis (Table 4) showed that relative density of *Culex* larvae is directly related to water temperature (B = 0.175, t= 2.588, r = 0.236, P =0.011) and reversely RH% (B = -2.695, t= -3.051, r = 0.316, P = 0.003).

Table 1: Numbers and percentages of surveyed aquatic bodies and habitats positive for *Culex pipiens* complex in Tabuk town, Saudi Arabia.

Type of habitats	No. of surveyed habitats	Habitats positive for larvae	
	No.	No.	%
Rain-filled pools	58	45	77.6
Leaking underground pumps	6	3	50.0
Water-cement tanks	10	0	0.0
Irrigated farms	12	0	0.0
Irrigated public gardens	13	0	0.0
Animal drinking water containers	9	0	0.0
Bird drinking water containers	8	0	0.0
Total	116	48	41.4

Fig 2: Types of mosquito larval habitats recorded in Tabuk town, Saudi Arabia (A: Rain-filled pools; B: Water-cement tank; C: Leaking underground pump; D: Irrigated farm and E: Irrigated public gardens; and F: Bird drinking water container).

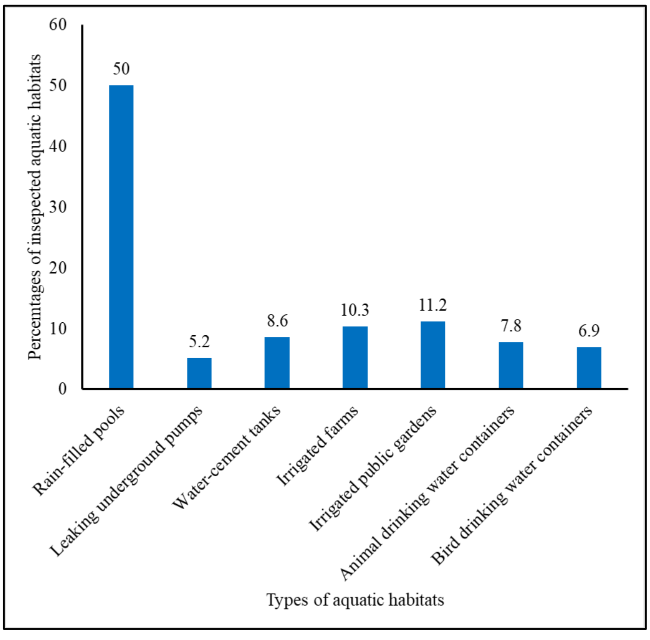

Fig 3: Percentage of a common type of aquatic habitats surveyed for mosquito larvae in the surveyed areas in Tabuk town, Saudi Arabia.

Table 2: Associations between the presence of *Culex pipiens* and *Cx. quinquefasciatus* larvae and habitat characteristics.

Characteristic	Chai-square	*P*-value
Type of habitats (Natural or man-made)	62.691	0.00
The permanence of habitats (Permanent or temporary)	8.578	0.003
Water current (Still or flowing)	0.194	0.660
Exposure to sunlight (Fully exposed, partially exposed or shaded)	0.712	0.399
Turbidity (Clear or turbid)	2.174	0.140
Substrate types (Mud, sand, or others)	7.725	0.005
Presence of predators (Present or absent)	5.258	0.022
Presence of grasses (Present or absent)	39.891	0.00
Presence of algae (Present or absent)	68.885	0.00

P value < 0.05 indicate significant difference.

Table 3: Comparison of some characteristics of aquatic habitats positive and negative for mosquito larvae in Tabuk town, Saudi Arabia.

Characters	Mean ± SE		*P-value*
	Yes*	No**	
Distance from houses	518.9 ± 48.8	339.5 ± 51.8	0.015
Size of water body in meter	7.37 ± 3.7	5.69 ± 0.79	0.605
Water depth in cm	6.42 ± 0.37	54.38 ± 12.7	0.002
Water temperature	23.80 ± 0.30	15.43 ±0.63	0.01
pH	8.28 ± 0.02	8.66 ± 0.09	0.00

P value < 0.05 indicate significant difference.
* indicates aquatic habitat positive for mosquito larvae.
** indicates aquatic habitat negative for mosquito larvae.

3.3. Larval relative density and distribution of positive habitats in Tabuk town

The overall density of larvae recorded in this study was 4.85 ± 0.490 larvae/dip. As shown in the figure, the density of mosquito larvae/dip was significantly higher in the rain-filled pools (5.12 ± 0.503) than that in habitats formed by leaking underground pumps (1.00 ± 0.00) (P = 0.00). Moreover, there was a significant difference in the relative density of larvae between the surveyed areas in Tabuk town (P = 0.00). The highest relative density of *Culex* larvae was recorded in the residential areas (10.20 ± 0.800) and the lowest in the industrial areas (1.0 ± 1.0) (Figure 4).

In this study, 23.9%, 20.0%, 78.0%, and 14.3% of the surveyed aquatic habitats in residential areas, industrial areas, valleys, and irrigated farms were positive for the presence of larvae, respectively (Table 4). The higher proportions of surveyed habitats were in residential areas (39.7%) and the valleys (35.3%) (Table 4). Of the total positive larval habitats recorded, more than 60% (66.7%) were in the valleys (Figure 5).

The results also showed that there was a significant difference between the different land use in numbers of the positive and negative habitats for the presence of mosquito larvae ($\chi 2$ = 35.576, P = 0.00; Linear-by-linear = 7.077; P = 0.008) (Figure 6).

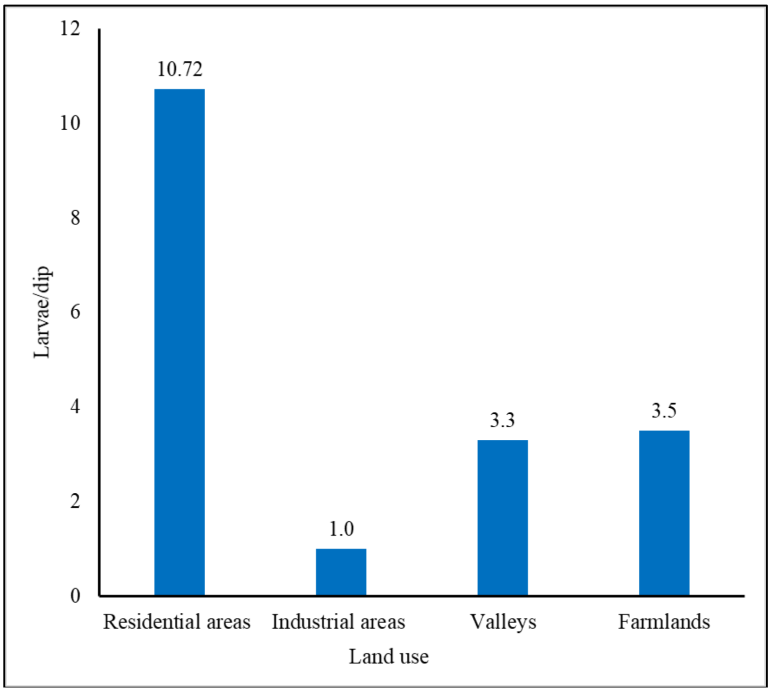

Fig 4: Relative density of mosquito larvae recorded in different environmental settings in Tabuk town, Saudi Arabia.

Table 4: Numbers and percentages of surveyed aquatic habitats and potential mosquito larval habitats in the surveyed areas in Tabuk town, Saudi Arabia.

Surveyed areas	No. (%) of surveyed habitats	Habitats positive for larvae	
		No.	%
Residential areas	46 (39.7)	11	23.9
Industrial areas	15 (12.9)	3	20.0
Valleys	41 (35.3)	32	78.0
Farmlands	14 (12.1)	2	14.3
Total	116	48	

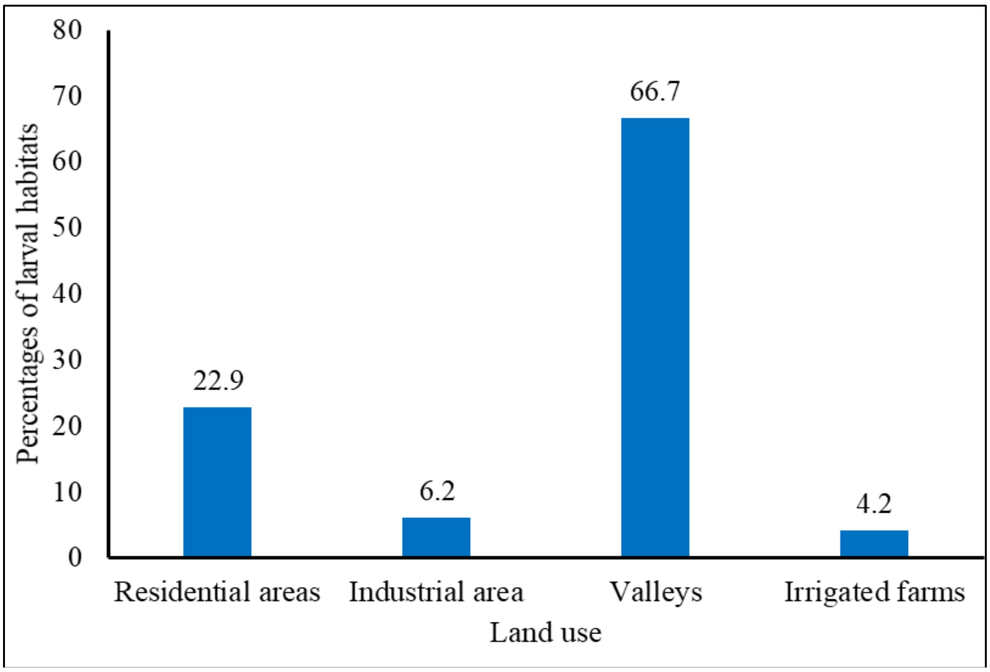

Fig 5: Percentages of aquatic habitats positive for mosquito larvae recorded in different environmental settings in Tabuk town, Saudi Arabia.

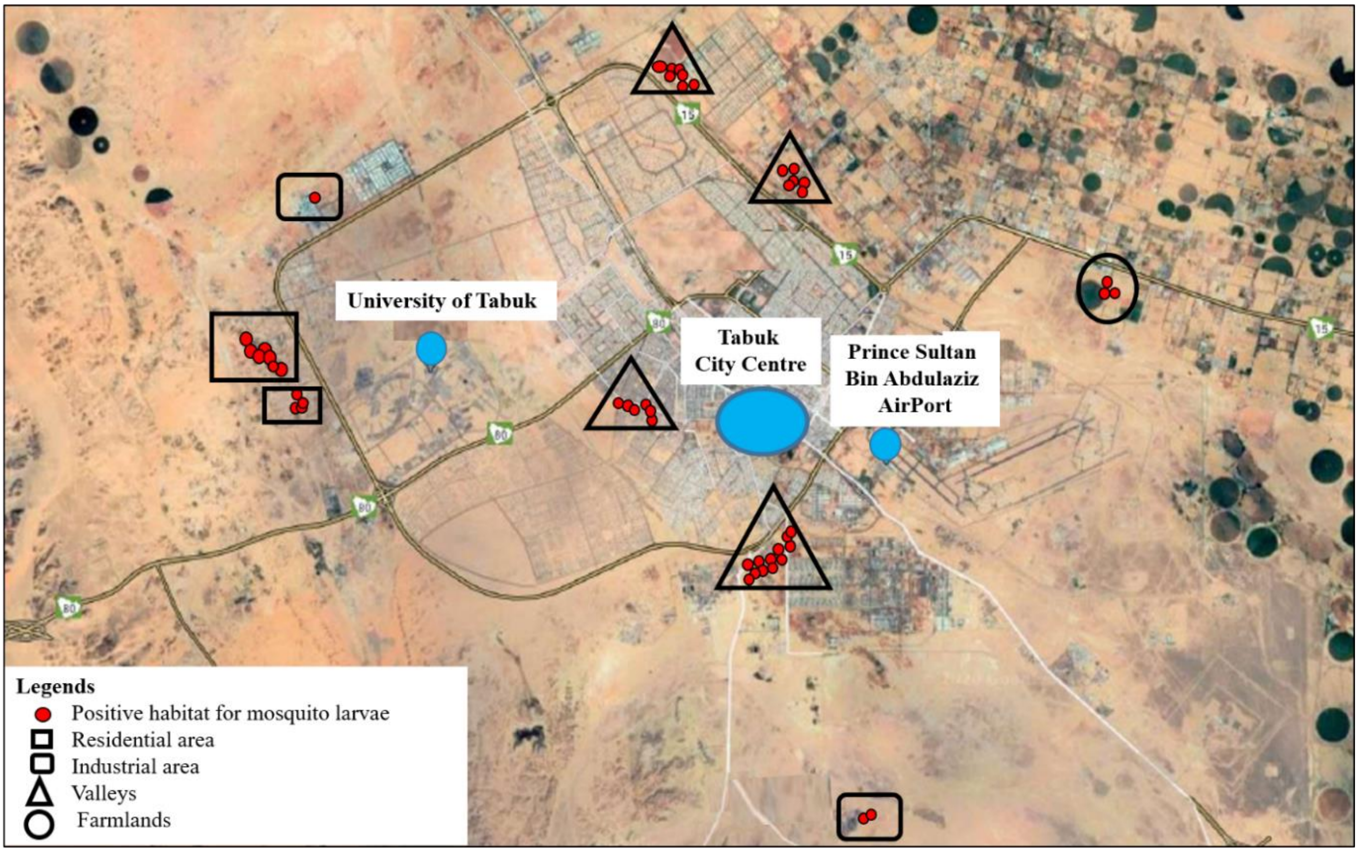

Fig 6: Distribution of positive habitats for mosquito larvae in different types of lands in Tabuk town, Saudi Arabia.

4. Discussion

Despite the huge effort to control mosquito-borne diseases, it has become a growing serious public health problem with a high global burden. This situation is due to the spread of mosquito vectors and/or pathogens into new areas as a result of global climate change [21]. Similarly, mosquitoes play an important role as vectors of several diseases in Saudi Arabian that include malaria [22], filariasis [23], Dengue fever [24], Rift Valley Fever [25], and West Nile Virus [17]. The control of mosquito-borne diseases through vector control has become more sounded and its success requires accurate knowledge of biology, ecology, and behavior of the mosquito vectors. This study was carried out to determine the species composition and the characteristic associated with *Culex pipiens* complex larval habitat in Tabuk town, Saudi Arabia. Importantly, this study is the first of its kind to characterize mosquito larval habitats in the Tabuk region.

Previous studies in Saudi Arabia showed the occurrence of several important vectors such as *Anopheles Arabiensis, Aedes aegypti*, and the *Cx. pipiens* complex [26]. The *Culex pipiens* complex species recorded in the country comprised two important species; *Cx. pipiens* Linnaeus 1758 (the northern house mosquito), *Cx. quinquefasciatus* Say 1823 (southern house mosquito) [26]. The larval collection in this study comprised two species; *Cx. pipiens* and *Cx. quinquefasciatus*. Both species are the most common mosquito species in Saudi Arabia [26]. *Culex pipiens* L. is considered a principal vector of introduced filariasis, WNV, and RVF in Saudi Arabia [26]. *Culex pipiens* was found infected with WNV in the Al-Madinah area [27]. The occurrence of both *Cx. pipiens* L. and *Cx. quinquefasciatus* in Tabuk town represent a risk of transmission of WNV as well as Bancroftian filariasis among the population living in this area.

The findings of this study also showed that larvae of *Cx. pipiens* (76.3%) were more dominant than those of *Cx. quinquefasciatus* in the surveyed areas in Tabuk town. This finding is in agreement with the results reported by Al Ashry *et al.* [18]. The authors found that larvae of *Cx. pipiens* were more common than those of other mosquito species collected in the Hail region at northwestern Saudi Arabia. Similarly, in two different larval surveys conducted in the Asir area in the southwest region of Saudi Arabia, larvae *Cx. pipiens* more dominant over than those of *Cx. quinquefasciatus* [18, 29].

Culex mosquitoes are known to breed in a diverse type of aquatic habitats where some species like *Cx. quinquefasciatus* can thrive in habitats rich with organic (i.e. polluted water) such as drainage canals and sewerage systems [30]. However, this study showed that rain-filled pools and leaking underground pumps are the main potential habitats for the breeding of mosquito in Tabuk town. The rain-filled pools formed 93.8% of the larval source in the surveyed areas. A study in Hail indicated that agricultural and building construction activities are the main source of mosquito breeding habitats in the area [18]. The variations in the findings between the two studies might be due to the difference in the study period and/or source of water surveyed for mosquito larvae in the two study areas. The current study was conducted as cross-sectional entomological surveys for seven months therefore, only defined types and number of water habitats were surveyed for mosquito larvae.

Details information on characteristics of the mosquito larval habitats is very crucial for developing and/or improving future vector control based and larval source management (LSM). Almost, mosquito breeding is influenced by some physical, chemical, and biological parameters of water habitats [31, 32]. In this study, larvae of *Cx. pipiens* and *Cx. quinquefasciatus* was significantly found to breed in temporary habitats characterized by shallow water with a mud bottom and, absence of vegetation (grasses), algae, and predators ($P < 0.05$). Although no significant differences were recorded, larvae of both species were found to thrive better in stagnant habitats with clear water (no solid wastes) and no shade (i.e. fully exposed to sunlight). The findings on the preference of *Cx. pipiens* for larval habitats characterized by stagnant, shallow clear water with a mud bottom and fully exposed to the sunlight was similar to the results of a study conducted in Cairo, Egypt [33]. In previous studies conducted in Hail and Asir regions of Saudi Arabia, *Cx. pipiens* was found to breed in habitats exposed to the sunlight; whereas, *Cx. Quinquefasciatus* in shaded habitats [18, 29]. However, Ashry *et al.* [18] found that habitats with stagnant water, presence of shade, algae, vegetation, and turbidity are the preferred breeding places for *Cx. pipiens*, and *Cx. Quinquefasciatus* in the Hail region.

It is known that water temperature and pH are important determinants that influence the growth, development, and survival of immature stages of mosquitoes [33, 34]. All the positive habitat recorded in this study for *Cx. pipiens* and *Cx. quinquefasciatus* were slightly alkaline (mean pH of 8.28 ± 0. 02) and had a mean water temperature of 23.80 ± 0.30 ºC. The results of linear regression analysis demonstrated that the relative density of *Culex* mosquitoes is directly related to temperature (P = 0.011) and inversely to relative humidity (RH%) (P = 0.003). These findings are in agreement with what was reported by a study on the western coast on *Cx. pipiens* [35]. Similarly, a positive correlation between water temperature and the relative density of *Cx. pipiens* and *Cx. quinquefasciatus* larvae were reported Hail region [18]. However, another study in the eastern region of Saudi Arabia indicated a negative correlation between the water temperature of the larval habitats and the relative density of *Cx. pipiens* and *Cx. quinquefasciatus* larvae [36].

The results also indicated significant differences in the larval densities between the types of habitats as well as between the surveyed areas (P = 0.00 for each). High larval density was significantly in rain-filled pools (5.025 ± 0.729 larvae/dip) as well as in residential areas (10.20 ± 0.800). These findings on larval density indicated that *Cx. pipiens* complex might prefer rainfall water than other types. It is known that the density of mosquito larvae in the aquatic habitats is highly influenced by different factors such as vegetation, temperature, pH, turbidity and, the concentration of different organic and inorganic compounds [37, 38]. For example, the presence of the vegetation in the larval habitats decreases larval density because it may cause barriers for egg-laying female, shadowing of the water surface, microbial growth as well as the occurrence of predators [39, 40]. The high density of *Cx. pipiens* and *Cx. quinquefasciatus* larvae in the residential areas might be due to that these species are common house mosquitoes (northern and southern house mosquitoes, respectively). These habitats are also more likely close to the source of blood meal for females and hence the engorge females would not travel a long distance to lay eggs. The occurrence of larval habitats with a high density of mosquito larvae in the residential areas might also represent a risk of future transmission of *Culex*-

borne diseases among the population of Tabuk town. A high proportion of positive habitats for mosquito larvae were recorded in valleys. This finding might be due to that valleys holds and retain rainwater for a long time and as shown in this study most of the positive habitats for mosquito larvae were rain-filled pools.

5. Conclusions

The occurrence of *Cx. pipiens* and *Cx. quinquefasciatus* might be a risk factor of transmission of WNV and bancroftian filariasis in the Tabuk area. Therefore, control of mosquito vectors is needed. The findings of this study indicate defined types of larval habitats in the area. Therefore, it would be cost-effective to conduct larval source management (LSM) to control and/or eliminate the mosquito populations from Tabuk town by treating the two main types of breeding habitats recorded in the area.

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