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Mosquito vectors survey reveals new record of *Culiseta subochrea* in Al-Ahsa Oasis, Saudi Arabia

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ABSTRACT

Objective: To determine the mosquito vectors prevailing in Al-Ahsa, eastern region, Saudi Arabia.
Methods: Monthly larval collections were conducted for one year by means of long dipper from different breeding sites in rural and semi-urban locations. Physical and chemical characteristics (pH, salinity and temperature of water) of positive sites were recorded.
Results: The survey revealed the presence of 7 mosquito species [*Aedes caspius, Anopheles multicolor, Culex perexiguus* (*Cx. perexiguus*), *Culex pipiens* (*Cx. pipiens*), *Culex pusillus, Culiseta langiareolata* and *Culiseta subochrea* (*Cs. subochrea*)] representing 4 genera. *Aedes, Ochlerotatus*, *Anopheles multicolor*, *Cx. perexiguus* and *Cx. pipiens* mosquitoes are known as important vectors. *Cs. subochrea* is recorded for the first time in Al-Hufuf Oasis. *Cx. pipiens* was the most common species followed by *Cx. perexiguus* then *Aedes caspius*. Results also showed that mosquitoes were abundant in winter and spring seasons then declined in summer and autumn seasons.
Conclusions: The present study is updating the total number of mosquito species of Al-Ahsa district, eastern province of Saudi Arabia by adding the newly record *Cs. subochrea* species. Furthermore, data obtained from the present study improve existing surveillance, assist in minimizing risk of disease transmission and develop vector management programs of the mosquitoes across this area.

1. Introduction

Mosquitoes are famous vectors of many diseases such as dengue, filaria, malaria, Rift Valley fever and Zika fever not only in Saudi Arabia but also worldwide. The most common diseases transmitted by mosquitoes in Saudi Arabia are dengue[1,2], filariasis[3], malaria[4,5] and Rift Valley fever[6,7]. Recently, malaria, Rift Valley fever and dengue fever are the most common mosquito-borne diseases endemic in different areas of Saudi Arabia[8]. In 1994 dengue virus was isolated for the first time in Jeddah city, western Saudi Arabia and the total confirmed cases raised to 319 during February 1994 to December 2002[9]. This number has been duplicated many times nowadays whereas 4411 cases of dengue was reported in 2013, with 8 cases of mortality representing four times higher compared to 2012[10]. All dengue cases were only reported in Jeddah and Makkah cities situated in western Saudi Arabia. The spread of the rift valley fever in Saudi Arabia in 2000 was the first ever to be recorded outside Africa and resulted in 76 death cases whilst 408 people were diseased[11]. A serological study for Rift Valley fever antibodies in Southwestern Kingdom of Saudi Arabia indicated positivity results among humans with exposure to ruminants[12]. Furthermore, mathematical models predicted high probability of rift valley fever occupancy in southwestern Saudi Arabia[13]. Haleem *et al.*[14] mentioned that three filarial cases were recorded from Saudi residences in Armed Forces Hospital, Riyadh in 2002 whilst WHO[15] reported that the number of indigenous malaria cases reduced by 88% in the period between 2006 and 2009.

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Literatures revealed that 26 mosquito surveys were conducted in Saudi Arabia. The majority of these 14 surveys were carried out in south western region which could be the results of the Rift Valley fever epidemic that happened in 2000 and the endemic of malaria in such region whilst 5 surveys were conducted in Eastern region, 4 surveys in the western region and 2 surveys in the middle region. The 5 mosquito surveys conducted in the eastern region focused mainly on Al-Ahsa[16-20]. The first three surveys[16-18] were quick collections for a very short time whereas the other couple of surveys[19,20] were conducted seasonally. Consequently, more accurate and reliable mosquito surveys are required to clarify the prevalent mosquito species in Al-Ahsa Oasis.

Therefore, the present study was carried out to identify mosquito vectors prevailing in Al-Ahsa Oasis, eastern region of Saudi Arabia. Such survey would be helpful in both planning and implementation of mosquito vector control strategies in this region.

2. Materials and methods

2.1. The study area

The present study was carried out in Al-Ahsa Oasis situated in the center of Al-Ahsa Province, eastern region of Saudi Arabia located at 25°23' N 49°35' E (Figure 1). It is one of the largest producers of date in the world. It is not only the largest Oasis in Saudi Arabia but also in the world. The climate is tropical and the temperature range is 9–28 ℃ in winter and 29–44 ℃ in summer. The average precipitation ranges are 0–30 mm depending on the season.

Figure 1. Map of Saudi Arabia showing Al-Ahsa Oasis.

2.2. Survey of mosquito larvae

Mosquito larvae population was surveyed in six localities (Al-Fudul, Al-Jafr, Al-Jishshah, Al-Tharah, Ar-Rumaylah and industrial city) representing rural areas except for the last location that represent semi urbanized area.

Monthly larval collections were conducted from different breeding sites during February 2010 to January 2011 within the assigned sites. Larvae were collected by handled net consisting of an iron ring (20 cm in diameter) and a muslin sleeve (30 cm long) attached to such ring. Three net dips were taken rapidly and gently from the surface of each breeding sites. Larvae were counted to determine their species prevalence.

The breeding sites were classified as either permanent or temporary aquatic bodies. The permanent bodies included stagnant and various sizes brackish pools, irrigation channels and drainage ditches. The temporary bodies comprised ground rain pools, cesspits and agriculture catchments. Short herbs were distributed in majority of the breeding sites whilst green algae were found in some. Some of these breeding places have high organic content due to the presence of animals' feces.

2.3. Physical and chemical characteristic of breeding sites

As well as mosquito samples, aquatic samples from breeding sites were taken to the laboratory for determining physical characters. Temperatures, pH and salinity were the physical characters that were measured and averages of these characters for the breeding sites within each locality were summarized in Table 1.

2.4. Larval identification

All larvae were collected with a pipette into large plastic containers, about one half liter, full of breeding site water to transport the larvae alive from the field to the laboratory. Then larvae were placed in alcohol 70% for killing and preserved in glass bottles for identifications. Larvae were examined and identified according to keys of Mattingly and Knight[16], Harbach[21], Azari-Hamidian and Harbach[22] and Al Ahmad *et al.*[23].

3. Results

The total number of mosquito larvae collected during the present study was 5 141 (Figure 2A) revealing the occurrence of 7 mosquito species (one aedine "*Aedes caspius* (*Ae. caspius*)", one anopheline "*Anopheles multicolor* (*An. multicolor*)" and 5 culicine "*Culex perexiguus* (*Cx. perexiguus*), *Culex pipiens* (*Cx. pipiens*), *Culex pusillus* (*Cx. pusillus*), *Culiseta langiareolata* (*Cs. langiareolata*) and *Culiseta subochrea* (*Cs. subochrea*)" species) in Al-Ahsa Oasis, eastern region, Saudi Arabia.

Cx. pipiens was the commonest species in Al-Ahsa Oasis comprising about 66.29% (3 408 larvae) of the total larval number (Figure 2A). It was collected from all localities (Table 2) except for Al-Jishshah and Al-Tharah that recorded high levels of water salinity (Table 1). This means that this mosquito breeds in low and/or moderate salinity aquatic bodies. The highest incidence of larvae was recorded in spring and winter seasons (Figures 2B and 3B) indicating that this mosquito is a cool weather species.

Cx. perexiguus come in the second order after *Cx. pipiens* and represent 14.51% (746 larvae) of the total larval collection (Figure 2A). As shown in Table 2, it was collected from 3 locations (Al-Fudul, Al-Jafr and Ar-Rumaylah). It was apparently more abundant in Al-Fudul (541/746) compared to Ar-Rumaylah and Al-Jafr (189 and 16/746 respectively). These places exhibited low and/or moderate water salinity which means that this mosquito species breeds in aquatic habitats with low and/or moderate salinity (Table 1). This species has one peak recorded in summer only (Table 2 and Figures 2B and 3B).

The third abundant species was *Ae. caspius* comprising 11.96% (615 larvae) of the total larval number (Figure 2A). It was collected from permanent or temporary brackish aquatic bodies from all localities except for Al-Tharah and industrial city (Table 2). These

Table 1

Physical and chemical measurements of breeding sites.

Date		Al-Fudul			Al-Jafr			Al-Jishshah			Al-Tharah			Ar-Rumaylah			Industrial city		
Year	Month	pH	Salinity	T	pH	Salinity	T	pH	Salinity	T	pH	Salinity	T	pH	Salinity	T	pH	Salinity	T
2010	February	6.48	0.22	25.0	6.96	0.56	24.7	6.72	0.74	23.3	7.67	1.65	26.5	7.09	0.77	23.0	7.52	0.31	21.0
	March	6.72	0.18	26.5	7.06	0.54	26.9	6.90	0.96	25.0	8.28	2.06	28.3	7.35	0.73	25.4	7.79	0.31	21.8
	April	7.11	0.19	27.8	7.49	0.49	28.1	7.43	0.88	26.0	7.11	1.51	29.0	7.41	0.73	26.9	7.79	0.31	22.6
	May	7.05	0.36	23.6	-	-	-	-	-	-	-	-	-	7.42	0.85	31.2	-	-	-
	June	6.76	0.30	25.6	-	-	-	-	-	-	-	-	-	7.11	0.89	26.5	-	-	-
	July	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	August	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	September	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	October	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	November	7.28	0.15	24.5	7.5	0.50	17.5	7.14	1.58	20.2	-	-	-	-	-	-	8.26	0.31	15.0
	December	7.06	0.28	16.7	7.41	0.54	15.4	6.72	0.82	16.3	-	-	-	7.43	0.89	15.3	7.96	0.34	11.3
2011	January	-	-	-	7.42	0.53	20.0	6.90	1.58	21.4	-	-	-	7.39	0.76	19.5	8.15	0.35	19.6

-: No measurements conducted due to dryness of breeding sites; T: Temperature.

Table 2

Frequency and distribution of mosquito larvae collected from different localities in Al-Ahsa Oasis.

Date		Al-Fudul				Al-Jafr				Al-Jishshah		Al-Tharah	Ar-Rumaylah						Industrial city
Year	Month	AM	AC	CP1	CP2	AC	AM	CP1	CP2	AC	AM	CP3	AC	AM	CP4	CP2	CL	CS2	CP2
2010	Feb	0	5	3	0	0	4	2	0	0	6	119	45	0	0	353	15	2	766
	Mar	0	53	1	22	-	-	-	-	-	-	-	6	19	0	549	23	7	421
	Apr	2	2	2	141	0	0	3	2	0	10	-	9	20	13	332	1	0	323
	May	2	1	228	1	0	-	-	-	-	-	-	66	20	38	22	0	0	-
	June	1	0	307	0	-	-	-	-	-	-	-	28	10	136	0	0	0	-
	July	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Aug	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sep	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oct	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Nov	-	-	-	-	4	97	11	0	-	-	-	-	-	-	-	-	-	42
	Dec	0	8	0	107	0	12	0	2	-	-	-	134	0	0	1	0	0	2
2011	Jan	-	-	-	-	4	0	0	0	139	0	-	111	2	2	290	0	0	32

-: No mosquitoes were collected due to dryness of breeding sites; AM: *An. multicolor*; AC: *Ae. caspius;* CP1: *Cx. perexiguus*; CP2: *Cx. pipiens*; CP3: *Culex pusilus*; CP4: *Culex perexig*; CL: *Cs. langiareolata*; CS: *Cs. subochrea.*

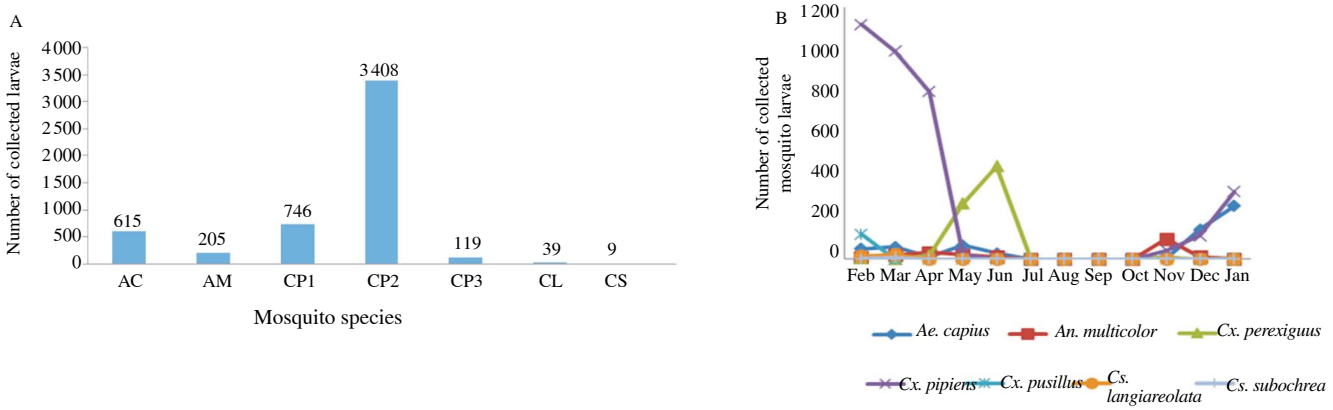

Figure 2. Frequency of mosquito larval species collected from Al-Ahsaa Oasis. AC: *Ae. caspius*; AM: *An. multicolor*; CP1: *Cx. perexiguus*; CP2: *Cx. pipiens*; CP3: *Cx. pusillus*; CL: *Cs. langiareolata*; CS: *Cs. subochrea*; A: Total number; B: Monthly prevalence.

aquatic bodies exhibited low to moderate salinity levels ranged from 0.15% to 1.58% and moderate pH levels ranged from 6.48 to 7.5 (Table 1) implying that this species prefers aquatic habitats with low to moderate and even high salinity levels. The incidence of Ae. *caspius* larvae was higher in winter and spring seasons than other seasons which means that this mosquito is a cool weather species (Figures 2B and 3B).

An. multicolor was the fourth mosquito species in the order and represent 3.98% (205 larvae) of the total larval number (Figure 2A). It was detected in permanent or temporary brackish aquatic bodies in Al-Fudul, Al-Jafr, Al-Jishshah and Ar-Rumaylah whereas low to moderate salinity levels ranged from 0.15% to 1.58% and moderate pH levels ranged from 6.48 to 7.5 (Table 1) indicating that this species prevails in sites with low to moderate salinity range. The

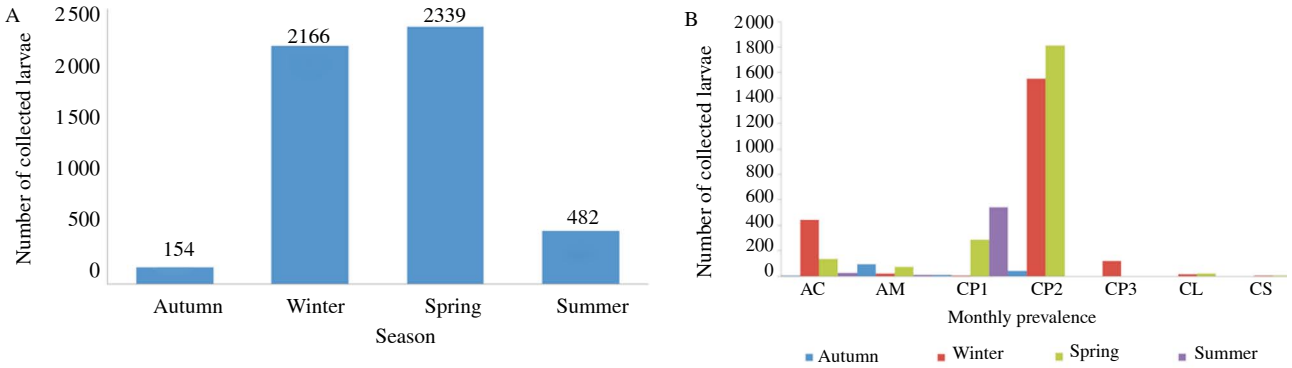

Figure 3. Seasonal abundance of mosquito larvae collected Al-Ahsaa Oasis. A: Total number; B: Monthly prevalence.

highest incidence number was recorded in autumn season (Figures 2B&3B).

Cx. pusillus larvae represented 2.31% (119 larvae) of the total encountered larvae (Figure 2A). All larvae were collected from one location, Al-Tharah, during one month only, February 2010, and have never been collected again since the pool dried out in May 2010. Salinity of such aquatic breeding site ranged from 1.56% to 2.06% but the pH ranged from moderate (7.11) to high level (8.28) (Table 1). This means that such mosquito species prefers aquatic bodies with moderate to high salinity levels.

Cs. longiareolata larvae were collected from one location only, Ar-Rumaylah, in few numbers comprising 0.75% (39 larvae) of the total larvae (Figure 2A). All larvae were collected during winter and spring (Figures 2B and 3B). Similarly, *Cs. subochrea* larvae were encountered in one location only, Ar-Rumaylah, in a very few number (9 larvae) comprising 0.17% of the total larvae collected (Figure 2A). Two larvae were collected in February and 7 larvae were collected in March 2010 (Table 2 and Figures 2B and 3B). This species was recorded for the first time in Al-Ahsa Oasis.

All collected larvae were coexisted together in the different breeding habitats except for *Cx. pusillus* that was solely collected from one location, Al-Tharah, during February 2010 and did not associated with any other mosquito larvae during the whole study. For seasonal abundance, number of collected mosquitoes larvae were higher in winter and spring seasons compared to autumn and summer seasons (Figure 3A).

4. Discussion

The present survey documented the prevalence of seven mosquito species (one aedine "*Ae. caspius*", one anopheline "*An. multicolor*" and 5 culicine "*Cx. perexiguus*, *Cx. pipiens*, *Cx. pusillus*, *Cs. longiareolata* and *Cs. subochrea*") in Al-Ahsa Oasis, eastern region of Saudi Arabia. These findings provide more mosquito fauna species to the previously mentioned surveys carried out in Al-Ahsa region.

The present survey indicated that some mosquito species were not recorded in previous mosquito surveys in AL-Ahsa. For example, *Cx. perexiguus* and *Cs. subochrea* were not recorded by Mattingly and Knight[16]. *An. multicolor*, *Cx. perexiguus* and *Cs. subochrea* were not among mosquito species collected by Wills *et al.*[18]. All the mosquito species that have been collected in the present study never been mentioned in the survey of Büttiker[17]. Additionally, the recent survey by Ahmed *et al.*[19] did not report the presence of both *Cs. longiareolata* and *Cs. subochrea* whilst Al Ahmed[20] did not detect *Cs. subochrea*. Such difference in findings among all these surveys could be due to either differences in surveyed locations or larval misidentifications alerting for relaying upon other more accurate and reliable methods such molecular identifications and/or cuticle hydrocarbons.

The most abundant mosquito species in the present study was *Cx. pipiens* (66.29% followed by *Cx. perexiguus* (14.51%), *Ae. caspius* (11.96%), *An. multicolor* (3.98%), *Cx. pusillus* (2.31%), *Cs. longiareolata* (0.75%) and *Cs. subochrea* (0.17%). Likely, *Cx. pipiens* was the most abundant and prevalent mosquito species in Asir Province (39% of all collected mosquito larvae), Western Saudi Arabia[24] and in Jazan Province (it was encountered in 90% of total collection sites), Southwestern Saudi Arabia[25,26]. *Cx. pipiens* is encountered in the majority of surveys conducted in Saudi Arabia. It has been recorded inthe eastern region[16-20], western region[26,27], southwestern region[24,25,28,29] and the middle region[30] of Saudi Arabia. In agreement with the present findings, it is commonly found in sites with low and/or moderate salinity[19]. Results also showed that this mosquito is a cool weather speciesas the highest incidence was in winter and spring. Similarly, Ahmed *et al.*[19] stated that winter season exhibited the highest incidence of larvae compared with the other three seasons in Al-Ahsa district, Eastern Saudi Arabia. In contrast, Abdullah and Merdan[28] mentioned that *Cx. pipiens* was relatively highly abundant during March to November. In respect to vectorial potential, *Cx. pipiens* is known as filarial vector in Saudi Arabia and other adjacent countries[3,21] and incriminated as vector of Rift Valley fever virus[21] and Sindbis virus[18].

Cx. perexiguus was the second common species in the present survey. It was collected from 3 locations out of 6 showing low to moderate water salinity implying that this mosquito species prevails in breeding habitats with low and/or moderate salinity. This species exhibited high incidence in summer season only. It was reported for the first time in Saudi Arabia by Harbach[21] and from that date *Cx. perexiguus* was introduced to the Arabian mosquito fauna. It was previously used to report it as *Cx. univittatus* which forming a complex consisting of 3 species (*univittutus, neavei* and *perexiguus*) [31]. *Cx. perexiguus* was reported for the first time from the Eastern Saudi Arabia by Ahmed *et al.*[19]. It has a limited distribution in Saudi Arabia since it has been reported in the middle region[30] and the eastern province[19,20]. *Cx. perexiguus* is involved in the transmission of filarial and arboviral diseases in humans[21].

Ae. caspius was the third abundant species in the present survey collected from all localities except for industrial city and Al-Tharah but with different abundances and high incidence in winter and spring seasons respectively. Like *Cx. pipiens*, it prevails in breeding sites with low and/or moderate water salinity. In agreement with

these results, Abdullah and Merdan[28] reported that larvae of this species were found in all months and became abundant during March-June in Asir region, Southwestern Saudi Arabia. Contrarily, it was the most abundant mosquito (65.66%) in a previous survey conducted in Al-Ahsa[19], inhibiting highly brackish water bodies (salinity ranged from 1.36% to 6.40%) but showed the same higher peaks in winter and spring seasons. Such mosquito is widely distributed in Saudi Arabia. It was encountered in eastern region[16-20], southwestern region[27,28,30] and western region[27,32]. *Ae. caspius* is vector of Riff Valley fever virus[33], Tahyna virus in the Mediterranean region and the West Nile virus[34].

An. multicolor was the only anopheline mosquito collected and was the fourth common species. Out of 5 previous mosquito survey in Al-Ahsa, 3 only reported this species[16,19,20] but the other 2 surveys[17,18] did not. Recent surveys in Southwestern[4,24,27,28,35] and Western Saudi Arabia[27,30] indicated that this species is among anopheline mosquitoes of Saudi Arabia. It is found in places vary in water salinity, was abundant in winter and with lower abundance than the other collected species. Likely, larvae of this species were collected during cold months in Asir region[28]. *An. multicolor* is regarded as a secondary malaria vector in Saudi Arabia[4].

Cx. pusillus was the fifth abundant species inhabiting brackish pools and was collected only from one location, Al-Tharah, during February 2010 then the pools dried out till the end of the study. A few studies[16,19,20] reported the occurrence of this species in Saudi Arabia in particular Al-Ahsa region as has been mentioned from the last two recent surveys. On the contrary, majority of the studies[17,18,21,25,27,28,30] did not record this species. These surveys indicated the limited distribution of this species in Saudi Arabia but with special tendency in prevalence to the eastern region particularly Al-Ahsa. In agreement with the present results, *Cx. pusillus* was collected from shores of salt lake "AL-Asfar" in Al-Ahsa, brackish pools and ditches whereas water salinity is high and showed high incidence in winter season[19].

Cs. longiareolata was encountered in one location only, Ar-Rumaylah, in a few numbers (total number of larvae = 39) during February to April 2010 and representing the sixth mosquito species collected in the present work. Out of 26 studies, some studies[16,18,20,24-26,28-30] mentioned the prevalence of this species in different places in Saudi Arabia but in variable percentages. It was the second commonest mosquito species collected from Asir province[24]. Out of 4 mosquito surveys conducted in Al-Ahsa, two surveys only[18,20] recorded *Cs. longiareolata* as well as the present work.

Like *Cs. longiareolata*, *Cs. subochrea* larvae were encountered in one location only, Ar-Rumaylah, in a very few numbers during February (2 larvae) and March (7 larvae) 2010. This species is a sister species to *Cs. longiareolata*, both collected together from the same location and same period. It is recorded for the first time in Al-Ahsa Oasis and was only recorded from Southwestern Saudi Arabia for the first time in 1995 by Abdullah and Merdan[28] meanwhile the present study is the second one to record such species in Saudi Arabia. New records of mosquito species still common in different regions in Saudi Arabia. For instance, *Culex wigglesworthi* is recorded for the first time from Asir Province, Southwestern Saudi Arabia[24]. *Aedes (Stegomyia) unilineatus* was recorded for the first time not only in Southwestern Saudi Arabia in 2003 but also outside its previous distribution in Africa, Pakistan, and India[36]. Furthermore, Both *Uranotaenia unguiculata and Mansonia* sp. collected from Al-Ahssa, Al-Khobar and Qatif, eastern province of Saudi Arabia by Wills *et al.*[18] and could be considered as new record at that era since they were not previously recorded among Saudi mosquito fauna. Additional new records to the mosquito species list happens not only in Saudi Arabia but also in neighbor Arabian Gulf countries whereas the most recent and prominent case is recording *Culex quinquefasciatus, Culex tritaeniorhynchus, Culex laticinctus, Culex sitiens* and *Cx. perexiguus* in Qatar by Kardousha[37]. The reason behind these new mosquito records in Saudi Arabia could be due to the fact that a few mosquito surveys conducted in such large country and in the same time such surveys cover neither all breeding habitats nor all locations. Likewise, missed-identification could increase or decrease number of the newly recorded mosquito species and alerting for avoiding such confusion by adopting accurate identification tools such as cuticular hydrocarbons andmolecular approaches. Such tools will assist in accurate identification, identifying both new records and new species and nominating members of species complexes as not all of them are vectors of diseases. Such latter information is insufficient in Saudi Arabia.

Figure 3 shows that majority of mosquito larvae and species were encountered during winter and spring seasons (2 166 and 2 339 larvae respectively) compared to autumn and summer (154 and 482 larvae respectively). Such results could be correlated with low to moderated aquatic bodies temperatures (Table 1) and the higher rainfall incidence in such periods. Similarly, precipitation and temperature were strong predictors for the distribution of *Culex tritaeniorhynchus* mosquito in Jazan Province, Southwestern Saudi Arabia[38]. *Aedes aegypti* larval abundance also showed significant temporal variation, being usually more abundant in wet season compared to dry one[26]. Recently, mathematical assessment of the role of temperature and rainfall on mosquito population dynamics revealed positive correlation[39].

The 7 recorded species in the present study is updating the last reports of Ahmed *et al.*[19] and Al Ahmed[20] for the total mosquito species of Al-Ahsa Oasis, eastern province of Saudi Arabia. *Cs. subochrea* was reported for the first time and the first 4 mosquito species was reported as vectors of human borne diseases whilst the other 3 species has no known medical influence. Information about bionomics of such vectors will assist in minimizing risk of disease transmission and outbreaks in Al-Ahsa Oasis as well as developing innovative integrated vector management strategies.

Conflict of interest statement

We declare that we have no conflict of interest.

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