ENTOMOLOGY

Identification of mosquito species and determination of population density in the Taif governorate, Saudi Arabia

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Abstract

The surveillance of mosquitoes is the primary method for protecting humans from the spread of dangerous diseases such as malaria and dengue fever transmitted by certain mosquito species. The present study aimed to survey and identify mosquito species in the Taif governorate to determine their medical importance and monitor their distribution across seasons. A black hole light trap was used to collect adult mosquitoes, with four readings per month over an entire year recorded. A total of 3796 adult mosquitoes were collected, and 12 species were identified. All species belonged to the following genera: *Culex, Aedes, Anopheles, Culiseta,* and *Lutzia*; however, mosquito genera of medical importance (*i.e.*, *Culex, Aedes,* and *Anopheles*) were examined in the present study. Adult mosquito species occurred throughout the entire year, with peaks of density and activity during summer and autumn (24-30°C), confirming that environmental temperature plays a key role in mosquito existence and distribution. This study might provide evidence regarding the spread of mosquitoes and creates a database of the mosquito species in the Taif governorate, which would assist in pest control programs.

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Introduction

Mosquitoes are the main vectors of various diseases to humans, such as malaria, dengue, filaria, and Zika fever (Pavela and Benelli, 2016). Mosquitoes can live in diverse environments and have an almost global reach (Rueda, 2007). Conducting surveys and investigating seasonal fluctuations of mosquito species, especially those of medical importance, are typical methods for mitigating risks to humans and animals caused by such species. The survey method collects essential information regarding the density of different mosquito species, and helps decrease the quantity of pesticides sprayed randomly in the environment (Mahyoub, 2011). The distribution and identification of mosquitoes have been studied by many researchers in Saudi Arabia. For example, Alikhan *et al*. (2014) and Al-Ghamdi *et al*. (2008) recorded four *Aedes* species and 11 *Anopheles* species, respectively, from Jeddah and its satellite town. Mattingly and Knight (1956) conducted a study covering the eastern, western, and southern regions of Saudi Arabia and recorded 25 species and sub-species, including *Aedes aegypti*, *Ae. arabiensis, Ae. caspius, Anopheles cinereus, An. coustani, An. fluviatilis, An. Sergentii, An. gambiae, An. multicolor, An. pharoensis, An. stephensi, An. pulcherrimus, An. turkhudi, Culex sitiens, Cx. pipiens molestus, Cx. pipiens fatigans, Cx. pusillus, Cx. sinaiticus, Cx. laticinctus, Cx. tritaeniorhynchus, Cx. tigripes, and Culiseta longiareolata.* Büttiker (1981) found that the most prevalent mosquito in Saudi Arabia was *Cx. pipiens.* Abdullah and Merdan (1995) recorded nine species of mosquitoes in the southwestern region of Saudi Aribia, including *An. multicolor, An. arabiensis,* and *C. subochrea.* El-Khereji *et al*. (2007) studied the spread of mosquitoes in Riyadh and recorded 15 species, including *Ae. caspius*, *An. stephensi*, *An. coustani*, *An. dthali*, *An. pretoriensis*, *Cx. laticinctus, Cx. perexiguus*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. simpsoni*, *Cx. sinaiticus*, *Cx. theileri*, *Cx. tritaeniorhynchus*, *Cx. univittatus*, and *C. longiareolata*. Nine species were recorded in Jeddah by Mahyoub *et al*. (2013), including *Ae. aegypti*, *Ae. caspius*, *Cx. quinquefas-*

ciatus, *Cx. tritaeniorhynchus*, *An. gambiae*, *An. sergentii, An. pharoensis*, and *An. dthali*. Alahmed *et al*. (2009) recorded 19 species belonging to four genera: *Culex*, *Aedes*, *Anopheles*, and *Culiseta,* with *Cx. arbieeni* reported for the first time in the Taif province.

Field studies have included monitoring the seasonal activity of mosquitoes. Reiter (2001) stated that the activity, behavior, and survival of mosquitoes and the mechanics of their transmission of pathogens are strongly influenced by climatic factors such as temperature, humidity, wind, and rainfall. A warm environment and high rainfall help mosquitoes to complete their life stages and accelerate the growth of pathogens carried by mosquitoes (Hales *et al*., 2002; Shaman and Day, 2007). In the Jeddah governorate, Al-Ghamdi *et al*. (2008) found that the seasonal abundance of *Aedes* mosquitoes in January was affected by an average temperature of 23°C and relative humidity of 55%, and showed a significant correlation between high relative humidity and increased mosquito population. Mahyoub *et al*. (2015) conducted another study in Makkah and these authors found that the mosquito genus with the highest density was *Culex* (95%) in February at a temperature of 25.6°C and relative humidity of approximately 54%, followed by *Aedes* and *Anopheles*.

The present study aimed to conduct a widespread survey of mosquitoes in the Taif governorate to identify mosquito species and determine the female-to-male ratio. In addition, this study aimed to investigate the effects of climatic conditions on the density and activity of mosquitoes.

Materials and Methods

Field study

The field study, conducted from January 2018 to December 2018 in the Taif governorate, revealed the presence of many mosquito species, especially those of medical importance, which were found continuously throughout the year.

Study area and survey of adult mosquitoes

The Taif governorate is one of the main tourist attractions in the Kingdom of Saudi Arabia, located in the southeast of the Makkah region on the eastern slopes of the Sarawat Mountains at an altitude of 1,700 m above sea level with coordinates of 21°16′30.34″N 40°24′22.16″E.

Adult mosquito samples were collected using black hole light traps (Bio-Trap, Gangnam-Gu, Seoul, 135–744, South Korea) once a week at a rate of four readings per month over an entire year. A total of 10 traps were used for the study, and two traps were placed at each site. The traps were hung at a height of almost 1.5 m from the ground, because these traps can be tampered with and their suction fan works more effectively when it is off the ground. The main feature of this trap is the metal part surrounding the fan, which is coated with titanium dioxide (TiO_2). The heat emitted from fluorescent lamps (ultraviolet rays) reacts with TiO_2 to produce carbon dioxide (CO_2), creating conditions similar to the human body and attracting mosquitoes close to the light trap. Ultimately, the mosquitoes were captured by the blow fan.

Five sites (central, north, south, west, and east) in the Taif governorate were selected to cover the majority of the study area, and represented different mosquito breeding sites, including parks, mosque toilets, drainages, marble factory, farms, brick factory, road bridges, and rainwater sites (Table 1). The traps were hung during the pre-sunset period (4-6 pm) and were collected the following morning (6-8 am), once a week for an entire year, with four readings per month. These times were chosen because they represent the active period of mosquitoes when they are searching for food. The trap containers were emptied of mosquitoes and placed inside independent plastic boxes, and labeled with the relevant data (site name and collection date). The samples were then taken to the Research Unit of Dengue Fever and Vector Control at King Abdulaziz University, where they were placed in a freezer for 1 h to kill the live insects before being removed and left at room temperature for 1 h to relax the muscles of the mosquitoes.

The mosquitoes were identified to genus and species using specialized taxonomic dichotomic keys (Barraud, 1934; Harbach and Knight, 1980; Reinert, 2000; Azzam, 2006). The samples were sorted by sex to determine the female-to-male ratio, which was calculated using Microsoft Excel 2010. Climate data, including temperature, relative humidity, and rainfall during the study period, were obtained from the General Authority of Meteorology and Environmental Protection. Pearson's correlation coefficient was calculated to determine the correlation between environmental factors and mosquito populations to calculate the influence of these factors on mosquito activity and to determine the active periods throughout the year in the Taif governorate.

The SPSS software program (version 25) was used for data analysis with one-way analysis of variance. Differences were considered significant at $p<0.05$. The relationships among temperature, humidity, rainfall, and mosquito density were determined using the Pearson's correlation coefficient.

Results

Survey and identification of adult mosquito species

A total of 3796 adult mosquitoes were collected across the study sites; these mosquitoes belonged to the main mosquito genera of vector diseases (*i.e*., *Aedes, Culex*, and *Anopheles*). A total of 12 mosquito species in the Taif governorate were classified based on their morphological characters. Two species belonged to the genus *Anopheles*, one species to the genus *Aedes*, and nine species belonged to the genus *Culex* (Table 2). Other species were found (*e.g., Lutzia* spp. and *Culiseta* spp.); however, these species did not have medical importance and were irrelevant to the present study.

Female-to-male ratio of mosquito density

The number of female mosquitoes collected by the light traps was higher than males for all genera during the study period, with 2856 females collected (75.23%) and 940 males (24.76%). The

Table 1. Coordinates of the mosquito study sites in Taif governorate using a GPS system.

Region	Location Description	Coordinates	
		N	E
North of Taif	Mall	21°21.346'	40°30.095'
	Mosque toilets	21°26.437'	40°30.067'
East of Taif	Workers' house	21°16.914'	40°26.618'
	Brick factory	21°16.872'	40°26.455'
Center of Taif	King Abdullah Park	21°16.344'	40°24.488'
	Butchery	21°16.370'	40°24.311'
West of Taif	Farm	21°20.492'	40°25.766'
	Old mosque	21°19.576'	40°26.749'
South of Taif	Marble factory	21°15.299'	40°27.501'
	Agricultural Nursery	21°14.576'	40°26.368'

differences between the sexes were highly significant in the genus *Culex* (p=0.0065) and the genus *Anopheles* (p=0.0415), whereas there was no significant difference between females and males in the genus *Aedes* (p=0.5762, Table 3).

Density of mosquitoes per months during the study year

Culex, *Anopheles*, and *Aedes* were present throughout the year; however, they were found at different rates. For the mean monthly population density values, there were significant differences for *Culex* (X=74.875±11.372, p=0.0001) and *Aedes* (X=3.395±0.763, p=0.0001), whereas for *Anopheles* (X=2.583±0.927), no significant differences were found (p=0.3912).

The study showed significant differences in the population density of genus *Aedes* during the year, with a peak intensity in November (X=15±1.95 with a significant difference (p<0.05), whereas the lowest values were recorded in February, April, and May (X=1±0.00). The study recorded a moderate population density for this genus during August, July, and September (7.5±3.013, 5±1.47, and 2.25±0.75, respectively), and a low population density during January, March, June, October, and December (1.75±0.47, 1.75±0.47, 1.75±0.47, 1.5±0.28, and 1.25±0.25, respectively, Figure 1 and Table 4). The results of the statistical analysis showed significant differences among months (p=0.0001).

The population density of *Culex* was higher than that of *Aedes* and *Anopheles*, representing 92.6% of the total collected species of medical importance during the study period. The highest population density was recorded in June (X=187±26.02), whereas the lowest population density was found in February (X=20±6.75). A moderate population density was recorded during August, September, and November, with highly significant differences between months (p=0.0001, Figure 2 and Table 4). *Anopheles* had the lowest population density of all three genera (3.29%), with its highest population density recorded in September (X=4.75±1.9), whereas the lowest density was recorded in February (X=1±0.00) and a moderate density was recorded during May, June, and November, with no significant differences between months (p=0.3912, Figure 3 and Table 4).

Seasonal activity and dynamic fluctuation of mosquitoes in the Taif governorate

The results showed the presence of genera of medical impor-

Table 2. Registered mosquito species in the Taif governorate during the study period.

No.	Genus	Species
1	*Aedes (Ae.)*	*aegypti*
2	*Culex (Cx.)*	*perexiguus* *theileri* *laticinctus* *poicilipes* *quinquefasciatus* *pipiens* *sitiens* *univittatus* *sinaiticus*
3	*Anopheles (An.)*	*dthali* *arabiensis*

Table 3. Comparison of the means and standard errors (SE) between male and female Culex, Anopheles, and Aedes mosquitoes in the Taif governorate.

Genus	Means±SE			
	Male (♂)	Female (♀)	LSD	P value
Culex	69.583±10.421^{a}	223.416±50.04^{b}	106.02	0.0065
Anopheles	3.5±0.75^{a}	7±4.30^{b}	3.352	0.0415
Aedes	5.50±2.22^{a}	7.5±2.73^{a}	7.31	0.576

*Means followed by the same letters in the same row showed no significant differences at p<0.05. Degrees of freedom = 22; LSD=Least significant difference.

Table 4. Population means and standard errors (SE) of adult *Culex*, *Anopheles*, and *Aedes* in the Taif governorate during the study period.

Month	Means±SE					
	Culex	*Aedes*	*Anopheles*	Temp. (°C)	Rh %	Rf mm
January	52.25±10.61^{cde}	1.75±0.478^{cd}	1.75±0.75^{abc}	14.75±0.629^{e}	48.5±3.926^{bc}	1±0^{b}
February	20±6.75^{e}	1±0.00^{d}	1±0.00^{c}	16.25±0.75^{e}	54.5±1.5^{ab}	1.75±0.478^{b}
March	35.50±4.83^{de}	1.75±0.478^{cd}	1.75±0 .478^{abc}	21±0.408^{d}	46.75±3.19^{bc}	1.25±0.25^{b}
April	35.75±11.49^{de}	1±0.00^{d}	2±0.577^{abc}	24.75±0.478^{c}	35.5±2.327^{de}	11.25±10.3^{ab}
May	82±11.60^{c}	1±0.00^{d}	3.25±1.314^{abc}	24.75±2.13^{c}	44.5±5.979^{cd}	13.5±7.55^{a}
June	187±26.02^{a}	1.75±0.478^{cd}	3.5±1.50^{abc}	31.25±0.478^{b}	22±1.08^{f}	1±0^{b}
July	65±9.69^{cd}	5±1.471^{bc}	1.5±0.50^{bc}	30.75±0.629^{b}	21.25±0.63^{f}	1.5±0.5^{b}
August	51.75±12.80^{cde}	7.5±3.013^{b}	1.75±0.478^{abc}	30.5±0.288^{b}	33.75±1.10^{e}	4±1.77^{ab}
September	55±9.83^{cde}	2.25±0.75^{cd}	4.75±1.93^{a}	37±0.408^{a}	60.25±5.71^{a}	2±0^{b}
October	118.75±8.34^{b}	1.5±0.288^{d}	2.5±0.645^{abc}	24.5±0.288^{c}	31.75±3.03^{e}	1.25±0.25^{b}
November	171±17.85^{a}	15±1.95^{a}	3±0.816^{abc}	24.5±1.55^{c}	46±3.31^{bc}	6.75±3.614^{ab}
December	24.5±6.66^{e}	1.25±0.25^{d}	4.25±2.136^{ab}	16.5±0.5^{e}	45.5±3.593^{bc}	6.5±3.403^{ab}
P value	0.0001	0.0001	0.3912	0.0001	0.0001	0.342

tance (*Aedes* spp., *Culex* spp., and *Anopheles* spp.) over the study year. However, the population density varied with climatic changes. The temperature affected the activity and intensity of mosquitoes. There was a significant positive correlation between the temperature and the different genera (r=0.351, p=0.0001), with the highest population density of *Aedes* spp. in November (X=15±1.95 0) that had a high temperature average (X=24.5±1.55°C). *Culex* spp. recorded the highest density in June (X = 187±26.02) that had a high temperature average (X=31.25±0.478°C). The lowest mosquito density was recorded in February that had an average temperature (X=16.25±0.75°C). *Anopheles* spp. recorded the highest density in September (X=4.75±1.93) at a high temperature average (X=37±0.408°C), whereas the lowest density was recorded in February that had a low temperature average (X=16.25±0.75°C) (Figures 4-7). However, the relative humidity and rainfall showed no significant effect on the population density of mosquitoes. There was a negative correlation with the value of r=−0.114 and r=−0.0469, respectively, with no regular increase in the number of mosquitoes noted when the relative humidity and rainfall increased compared to temperature (Table 4).

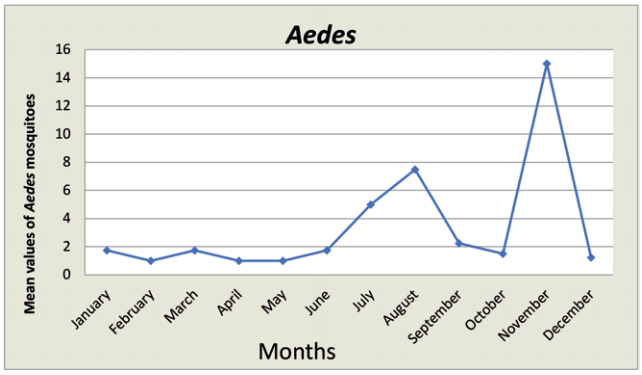

Figure 1. Mean values of *Aedes* mosquitoes during the study period in the Taif governorate.

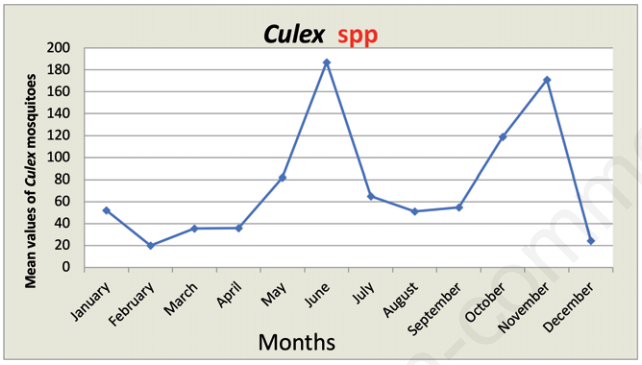

Figure 2. Mean values of *Culex* mosquitoes during the study period in the Taif governorate.

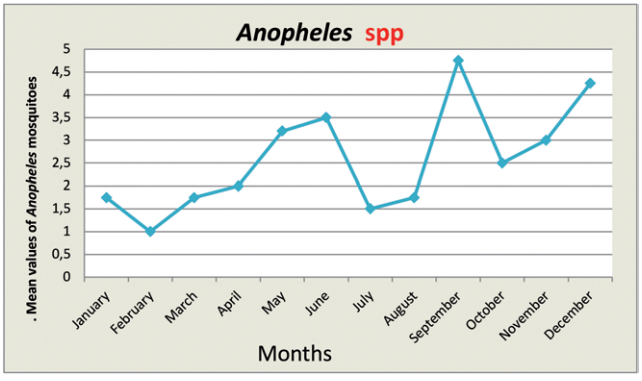

Figure 3. Mean values of *Anopheles* mosquitoes during the study period in the Taif governorate.

Discussion

In the present study, black hole light traps were used to collect adult mosquitoes. This trap has greater effectiveness in catching mosquitoes than other traps such as CDC miniature light traps (Clarke Mosquito Control, Roselle, IL), which require dry ice around the trap as the CO_2 source and need to have their batteries charged. The black hole light trap is an eco-friendly trap and it has proven effectiveness in catching mosquitoes from previous studies (Al-Ghamdi *et al*., 2008; Alikhan *et al*., 2014). A total of 3796 mosquito adults were collected, and 12 species were identified in the Taif governorate in the present study. These species included *Cx. perexiguus*, *Cx. theileri*, *Cx. laticinctus*, *Cx. poicilipes*, *Cx. quinquefasciatus*, *Cx. pipiens*, *Cx. sitiens*, *Cx. univittatus*, *Cx. sinaiticus*, *An. dthali*, *An arabiensis*, and *Ae. aegypti*. *Culiseta* spp. and *Lutzia* spp. were also present. The three genera *Aedes*, *Culex*, and *Anopheles* are of greater medical importance in Saudi Arabia than genera *Lutzia* and *Culiseta*. However, *Culiseta* spp. does have veterinary importance and can transmit other diseases to humans, such as the encephalitis virus, in other parts of the world (Edman *et al*., 1972).

The species found in the present study are similar to those found in previous field studies. For example, Alahmed *et al*. (2009) recorded 19 species, Mahyoub *et al*. (2013) recorded nine species in Jeddah, located northwest of Taif Therefore, all species recorded in the present study have been found in other regions of the Kingdom. However, it was essential to perform this survey to iden-

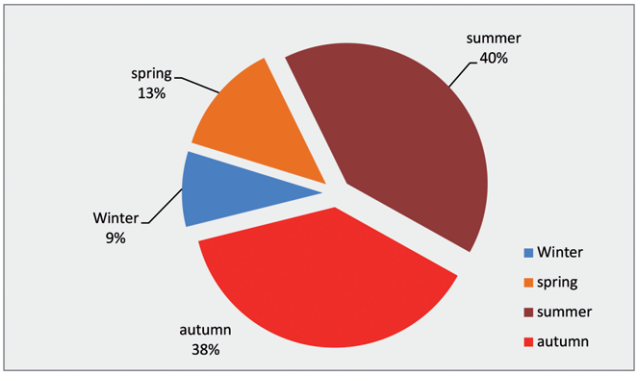

Figure 4. Percentage of seasonal population density of mosquito species of medical importance in the Taif governorate.

tify the species of medical importance, such as *A. aegypti*, which is considered the main vector of dengue fever, and such identification will help in future mosquito control and management programs.

The increase in species density was due to varying environments and weather conditions. The most abundant genus was *Culex*, which comprised 92.6% of the total collected mosquito species during the collection period. The survey results showed that the ratio of mosquito females (75.23%) collected by light traps was higher than that of males (24.76%). Similar observations were recorded in another study conducted in the Baljurashi governorate when a black hole light trap was used (Alghamdi, 2018). The percentage of females collected by Clark traps was approximately 98% in a field study conducted in the Jeddah governorate (Alqhtani, 2009). CDC light traps showed that the female-to-male ratio was 15.7:1 during a study conducted in the city of Makkah (Khater *et al*., 2013). The black hole trap is designed to produce CO_2, which is important for the attraction of female mosquitoes. Females travel great distances from breeding sites seeking hosts during the day, making them more likely to fall into traps. However, the black hole trap can also catch males, which is attributed to the presence of a fluorescent lamp, nearness of the trap to breeding sites, and males flying with females around the trap for reproduction. These observations are in agreeance to other studies that were performed in Riyadh, Makkah, Madinah Al munwwrah, Najran, Jeddah, and the eastern region of Saudi Arabia (El-Khereji *et al*., 2007; Alahmed *et al*., 2009; Alahmed *et al*., 2010; Kheir *et al*., 2010; Mahyoub *et al*., 2013; Alahmed *et al*., 2015).

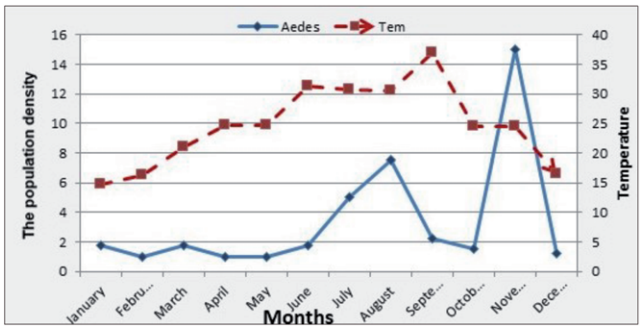

Figure 5. Relationship between temperature means and population density of adult *Aedes* during the study period.

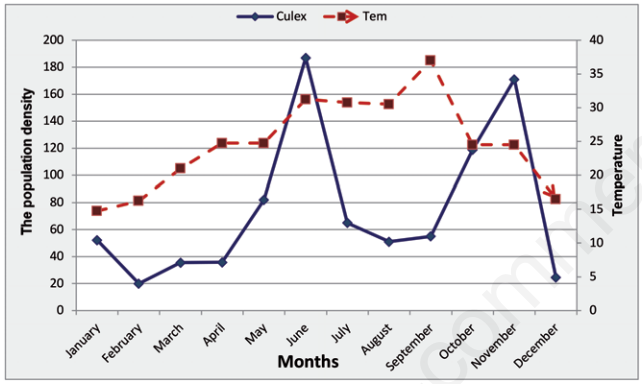

Figure 6. Relationship between temperature means and population density of adult *Culex* during the study period.

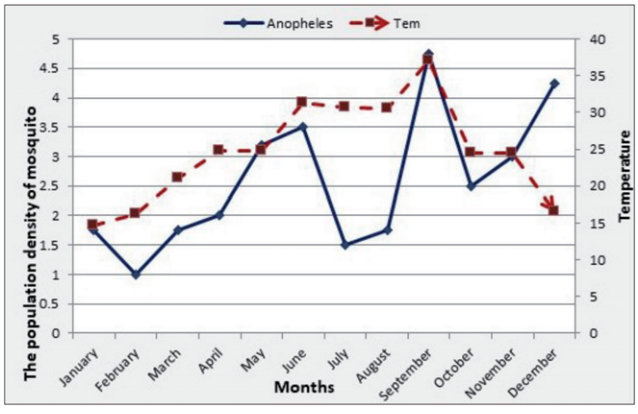

Figure 7. Relationship between temperature means and population density of adult Anopheles during the study period.

Temperature had a more significant effect on the presence of mosquitoes than other climatic conditions. A peak in density and activity was observed when the temperature was between 24 and 37°C, corresponding to temperatures recorded during previous studies in several regions of Saudi Arabia (Al-Ghamdi *et al*., 2009; Alahmed, 2012; Hassan *et al*., 2017, Khan *et al*., 2018). There was an increase in activity and density of mosquitoes during summer and autumn in the present study, which was consistent with the study by Soltani and Keshavarzi (2016) conducted in Iran, and showed the highest mosquito density recorded in July. Relative humidity did not significantly affect mosquito density, which might be due to its association with temperature, *i.e*., when the temperature decreased, there was usually a large increase in the relative humidity. This result did not correspond with previous studies, which found that the increase in relative humidity in the coastal and warm areas of the Kingdom, especially during summer, played an important role in increasing the density, activity, and mating of mosquitoes. This difference might be due to the role of the relative humidity in moistening the air and decreasing the temperature, thereby protecting mosquitoes from lethal temperatures (Yamana and Eltahir, 2013; Al-Ghamdi *et al*., 2009).

The rainfall rate did not significantly influence the collection of the highest number of adult mosquitoes, although rain does play an important role in mosquito breeding and reproduction (Alahmed, 2012). However, during higher rainfall in the present study, adult mosquito activity decreased at all study sites, and the opportunity to catch mosquitoes in the light traps was limited. These environmental conditions might vary between field studies. The monitoring and identification of mosquitoes performed during the present study will help develop pest control programs and limit the transmission of diseases to the Taif governorate residents.

Conclusions

The present study is the first comprehensive field study of adult mosquito species, focused on the Taif governorate and mosquito species belonging to five genera, *Culex*, *Aedes*, *Anopheles*, *Culiseta*, and *Lutzia*, were recorded. *Cx. poicilipes, Cx. sitiens, An. arabiensis, and Lutzia* spp. were recorded for the first time in the Taif governorate. The highest density of mosquitoes was observed during summer and autumn, with the temperature playing a primary role in the

spread of mosquitoes. Molecular techniques should be applied in future studies to accurately identify sibling species and morphological methods performed to contribute to the success of the control plan and reduce serious diseases caused by mosquitoes.

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