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Biodiversity of Culicidae (Insecta: Diptera) in the Region of Khenchela (Northeast Algeria)

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BIODIVERSITY OF CULICIDAE (INSECTA: DIPTERA) IN THE REGION OF KHENCHELA (NORTHEAST ALGERIA)

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ABSTARCT

Mosquitoes are considered as vectors of serious human diseases. The prevention of parasitic and arboviral vector-borne diseases is based mostly on vector control strategies. This control cannot be effective unless a strong knowledge of mosquito biology and distribution in the working area. In this context, and in order to improve the knowledge on the specific diversity of the culicids fauna in the region of Khenchela (northeast of Algeria), an inventory of mosquitoes was carried out for the first time in this region. Larval collection was conducted in various types of habitats through continuous monitoring of seven sites from May 2020 to September 2021.Systematic study revealed the presence of 10 species belonging to 5 genera; including *Culexpipiens* (Linneaus, 1758), *Culexterritans* (Walker, 1856), *Culextheileri* (Theobald, 1903), *Culexmodestus* (Ficalbi, 1890), *Culisetalongiareolata* (Macquart, 1838), *Culisetaannulata* (Schrank, 1776), *Aedesgeniculatus* (Olivier, 1791), *Aedesvexans* (Meigen, 1830), *Orthopodomyiapulcripalpis* (Rondani, 1872) and *Uranotaeniaunguiculata* (Edwards, 1913). The species *Cx. pipiens* was the most abundant (44.26 %) followed by *Cs. longiareolata* (39.57 %). The variation of diversity in the different sites depends on the type of breeding habitat. Our findings provide very important information on biodiversity, distribution and the relationship between species abundance and ecological factors. Therefore, the results can be very useful for the design and implementation of effective control programs.

KEYWORDS: Inventory, biodiversity, mosquitoes, humidity, Khenchela.

INTRODUCTION

Mosquitoes or culicids are a group of insects that have an important role in the spread of diseases. These insects have holometabolous development including aquatic larvae and aerial adults (Dahchar et al., 2016). These hematophagousDiptera occupy a particular position because of the considerable nuisance they can cause (Becker et al., 2010). On the other hand, Culicidian species have a behavior that differs from one region to another, which influences their vectorial role (Hassaine, 2002). Mosquitoes are considered vectors of several severe human diseases such as malaria, yellow fever, West Nile virus, dengue, Chikungunya and Zika. In Algeria, Culicidae are the most harmful biting insects for the human population and keep transmitting infectious diseases (Tine-Djebbar et al., 2011). According to the responsible of malaria monitoring at the National Institute of Public Health in

Algeria (INSP), 2726 cases of malaria were recorded in 2020, resulting in three deaths, stating that all cases were imported (WHO, 2021).

Continuous evolution of the geographical distribution of certain species, appearance of new species or the disappearance of others, under the impact of local modifications (environmental management, increase of hydraulic projects and extension of urbanization...), or even general changes (global warming), can constitute the origin of the re-emergence or the emergence of vector-borne diseases (FilaliMouatassem et al., 2019).

In order to carry out an efficient vector control, it is necessary to have a good knowledge of the mosquito population in terms of their species diversity and ecological characteristics (Manguin and Boëte, 2011; Li et al., 2019). In Algeria, several studies have been made in different regions including Algiers, Annaba, Tizi Ouzou (Lafri et al., 2014; Arroussi et al., 2021; Chahed et al., 2021). Nevertheless, no such study has been carried out in the province of Khenchela (northeastern Algeria). For this reason, the present study aims to conduct an inventory of mosquito species present in this region, for the first time, in order to better understand their diversity that can be used to design an integrated vector control program, characterized by targeted and cost-effective intervention.

MATERIALS AND METHODS

Study Area

The region of Khenchela is located in the northeast of Algeria (6°32’ and 7°34’ East longitude and between 35°7’ and 35°38’ North latitude, altitude of 1200 m). The city is bordered by the province of Oum El Bouaghi in the north, the province of El Oued and Biskra in the south, the province of Tebessa in the east and the province of Batna in the west (Figure 1).

The region of Khenchela is characterized by a semi-arid climate, with a hot and dry summer recording a maximum temperature of 34.9 °C during July, and a very cold winter with an average minimum temperature of 1.85 °C in January. The annual average rainfall is about 508.83 mm (Drouai, 2018).

Sample Collection and Culicidae Identification

Sampling was performed on fortnightly basis over a period of 17 months (May 2020 to September 2021), between 09:00 and 11:00 o’clock. Fifteen natural and artificial breeding sites were chosen (Figure 2). The artificial breeding sites were represented by basin, tank, tire, cellars, metal barrel, pit, bucket, well, jar and sewage leaks; whereas the natural breeding sites were constituted by pond, dam, water retention of rain, wadi and tree hole. These sites are distributed over seven stations of the region including Khenchela, Bebar, Ain Djerboue, Oued Elhatiba, El Mahmel, Ouled Amara and OuledAzzedine (Table 1). The choice of sampling sites depended on the location, the presence of potential larval sites and diversity of environments.

The sampling technique consists of using a 500 ml ladle (dipper method) that has been plunged several times (We focused our identification on the larvae of mosquito only, because we kept the adults for another part of the study (to make further analyses on them)). Then, the collected larvae were placed in plastic bottles filled with heeling water, hermetically sealed and labeled (indicating the date of sampling and station),

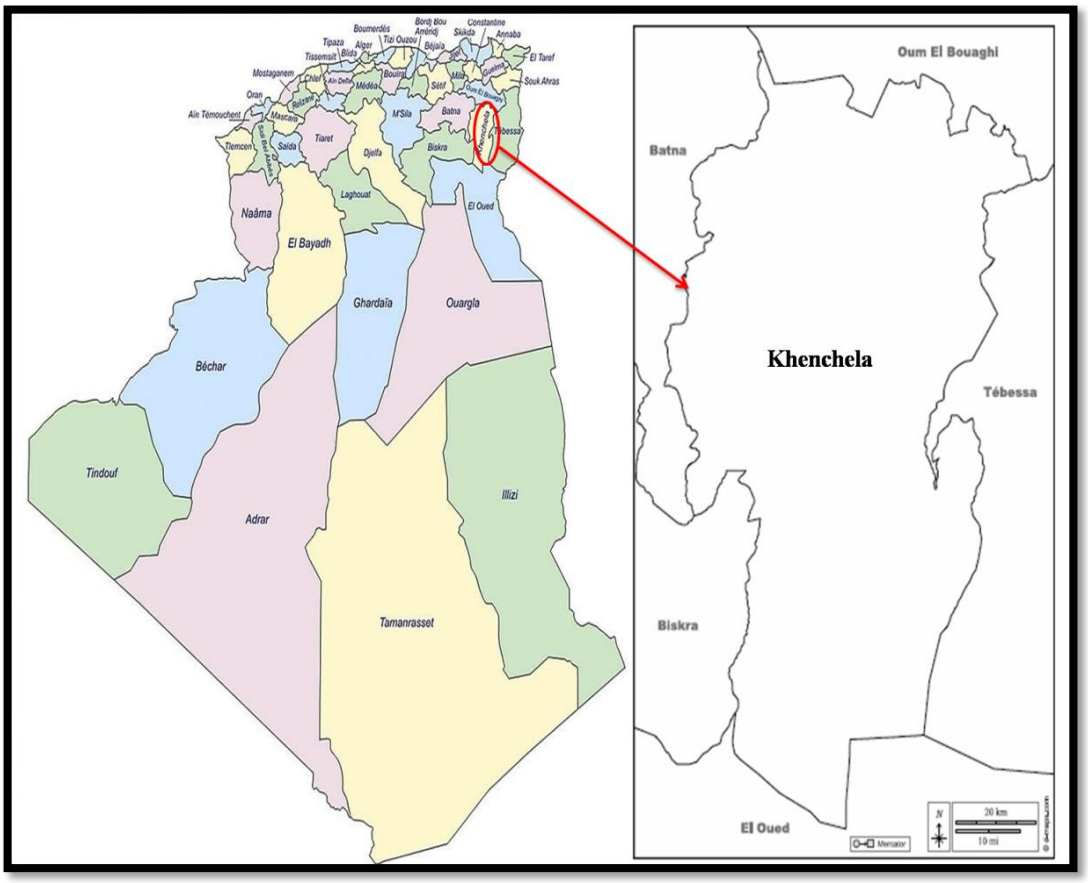

Figure 1: The geographical location of Khenchela region (Algeria)

Table 1: Geographic characteristics of mosquito larval collection sites in Khenchela region

Site name	Habitat types	Latitude (N)	Longitude (E)
Khenchela	Urban Peri-urban	35°27’16’’	7°10’22’’
Bebar	Urban Agricultural	34°45’39’’	7°00’01’’
AinDjerboue	Rural	35°14’55’’	7°07’22’’
OuedElhatiba	Rural	35°12’48’’	7°08’14’’
El Mahemmal	Urban Peri-urban	35°20’24’’	7°15’36’’
Ouled Amara	Rural	35°21’53’’	7°19’56’’
OuledAzzedine	Rural	35°18’55’’	7°13’19’’

The bottles were carefully and directly transported to the laboratory and the specimens were decanted in plastic cups in order to separate the larvae from the debris. The larvae were maintained in plastic containers with the water of mosquito shelter, and having the dimensions of 30 cm x 30 cm (width x length).Then, these containers were put in room with temperature 25 °C, humidity 70 % and 12 hours of light. The larvae were fed with cat kibble and the surface of the water was cleaned daily. The rearing water was changed to avoid death of specimens by asphyxiation caused by the decomposition of food in water. The

change of water was performed using a Pasteur pipette and the larvae were placed in plastic cups containing dechlorinated tap water (Rehimi and Soltani, 1999)

Figure 2: Mosquito breeding sites (Shot HALIMI I)

Only the 4th instar larvae were recovered and taken into account for the slide mountings. The 4th instar larvae were identified by their chetotaxy. The L4 larvae are larger than the L3. In addition, they have body subdivided clearly into three distinct parts (head, thorax and abdomen) (Karida et al., 1998; Merabti, 2016). They were collected by a Pasteur pipette and stored in Eppendorf tubes in a 70 % ethanol solution (Benmalek et al., 2018). The method of sample preparation and mounting is based on the protocol proposed by Matile (1993). Briefly, the larvae were cleaned in a warm 10% KOH solution. Afterwards, they underwent 2 baths with distilled water, and they were then placed directly in absolute alcohol for 3 minutes. The larvae were mounted under a light microscope with a drop of Canada balsam to fix them between the slide and the cover glass. Specimens were identified using dichotomous keys of Himmi et al. (1995) and the results were then confirmed by the identification software of The Mosquitoes of Mediterranean Africa (Bruhnes et al., 1999) and Moskey Tool (Gunay et al., 2018).

Data Analysis

The exploitation of our results was performed by ecological indices of composition and structure: relative abundance, frequency of occurrence, Shannon-Weaver. To investigate a possible correlation between species abundances and the three measured climatic factors (mean temperature, mean rainfall and mean humidity), we calculated Spearman's rank correlation with a significance level of α = 0.05 % using GraphPad Prism 5 Demo software.

RESULTS

A total of 4144 specimens were collected from various sites (natural and artificial) throughout the province of Khenchela.

The composition of the inventoried culicid fauna in this region revealed the presence of 10 species belonging to a single subfamily "Culicinae" divided into 5 genera: *Culex, Culiseta, Aedes, Orthopodomyia* and *Uranotaenia*.

Table 2: List of Culicidaecollected in Khenchela region, Algeria

Family	Subfamily	Genus	Species	Medical importance
Culicidae	Culicinae	*Culex*	*Culexpipiens* (Linneaus, 1758)	Yes
			Culex (Neoculex) territans (Walker, 1856)	No
			Culex (Culex) theileri (Theobald, 1903)	Yes
			Culexmodestus (Ficalbi, 1890)	Yes
		Culiseta	*Culiseta (Allotheobaldia) longiareolata* (Macquart, 1838)	Yes
			Culisetaannulata (Schrank, 1776)	No
		Aedes	*Aedes (Finlaya) geniculatus* (Olivier, 1791)	No
			Aedesvexans(Meigen, 1830)	Yes
		Orthopodomyia	*Orthopodomyiapulcri palpis*(Rondani, 1872)	No
		Uranotaenia	*Uranotaenia (Pseudoficalbia) unguiculata*(Edwards, 1913)	Yes

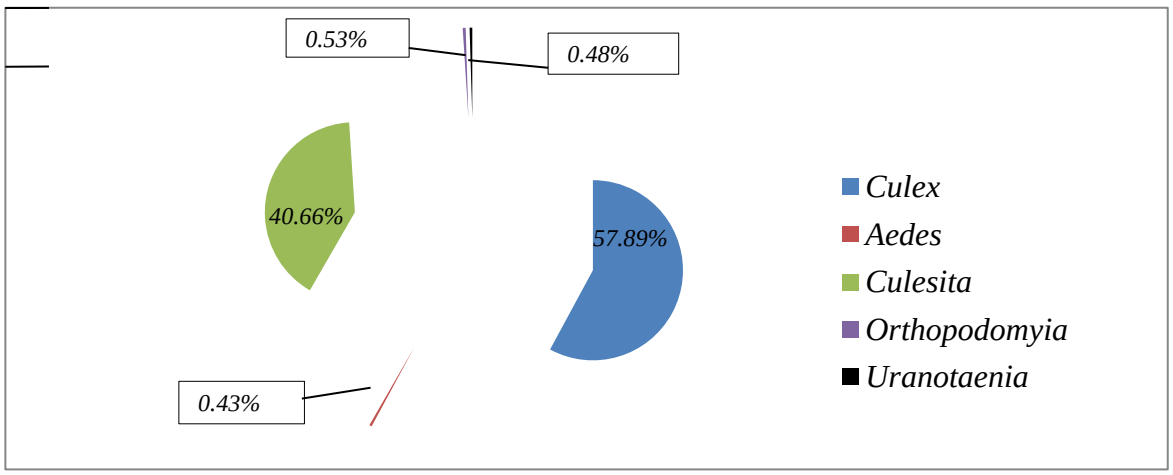

Figure 3: The percentages of frequency of mosquitoes genera caught in Khenchela region, Algeria.

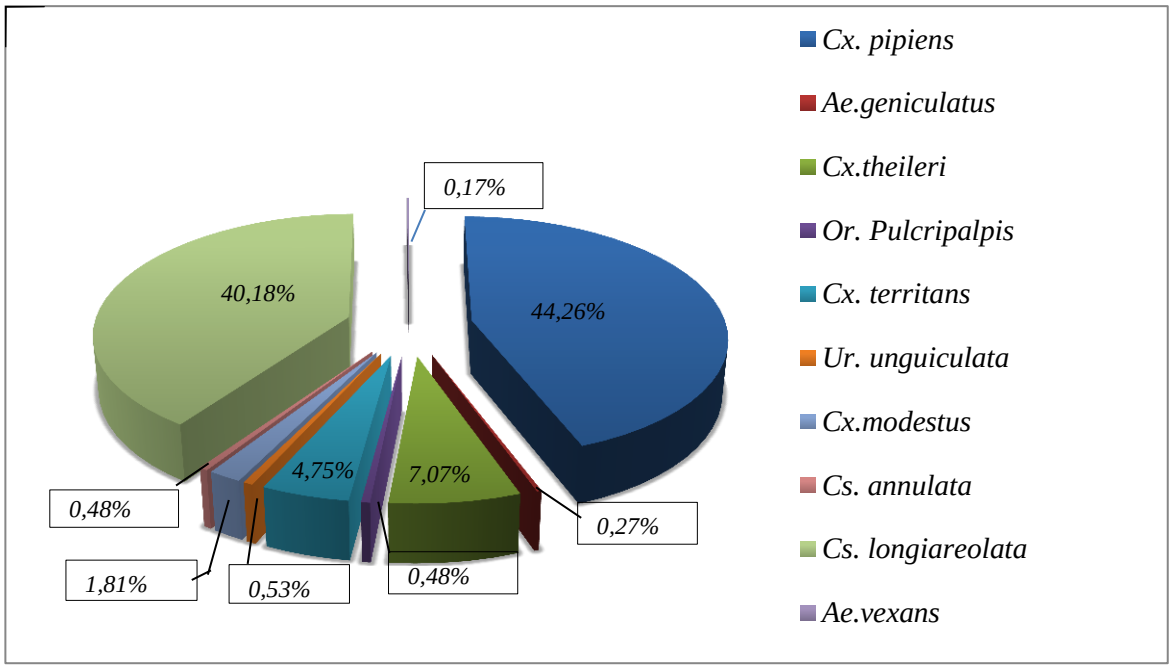

Figure 4: Diversity and relative abundance of mosquito species recorded in the study area (from May 2020 to September 2021).

The genus *Culex* occupies the first position regarding the number of species, being represented by 4 species: *Culexpipiens*, *Cx. theileri*, *Cx. territorans*and *Cx. modestus*. Whereas, the genus *Culiseta* and *Aedes* represented by 2 species: *Culesitaannulata*, *Cs. longiareolata* and *Aedesgeniculatus* and *Ae. vexans* respectively. The genera *Orthopodomyia* and *Uranotaenia* represented by one species each *Or. Pulcripalpis* and, *Ur. unguiculata* respectively Table 2.

Concerning the number of collected specimens (Figure 3), the genus *Culex* was the most frequent with a total of 2399 specimens (57,89 %) followed by genus *Culiseta* with 1685 specimens (40,66 %). Genus *Orthopodomyia* (22 specimens), *Uranotaenia* (18 specimens) and *Aedes* (18 specimens) were poorly represented with a percentage lower than 1 % for each.

The most abundant species in the genus *Culex* was *Cx. pipiens* (44,26 %), followed by *Cs. longiareolata* (40,18 %) while *Cx. theileri, Cx. territorans*and *Cx. modestus* were quite rare, the other species: *Ur. unguiculata*, *Or. pulcripalpis*, *Cs. annulata*, *Ae. geniculatus* and *Ae. vexans*were poorly represented (Figure 4).

Concerning the frequency occurrence of the collected species, *Cs. longiareolata* (39,57 %) and *Cx. pipiens*(37,87 %) are considered as accessory species, while the species *Cx. territorans*, *Cx. theileri*, and *Cx. modestus* can be considered as accidental species. However, *Or. pulcripalpis*, *Ur. unguiculata*, *Cs. annulata*, *Ae.geniculatus*and *Ae. vexans* are rare species (Table 3).

The distribution of the Culicidian fauna in the different sites in the Khenchela region during the study period is shown in Figure 5. It can be seen from the Figure 6 that the first peak was in May and June and the second highest in August and September. The effect of humidity and temperature on the spread of mosquitoes is very evident in our graphical presentation. The statistical study shows that species abundance was strongly positively and negatively correlated with temperature (r= 0.75; p= 0.0005) and humidity (r= -0.86;

p= 0.0000083) respectively, while no significant correlation was found between ainfall and species abundance p> 0.05 (Table 4).

Table 3: Ecological parameters of composition of the Culicidian populations in the study area

Species	N	FC %	Occurrence	C %	Category
Cx. pipiens	1834	44,26	89	37,87	Accessory
Cx. theileri	293	7,07	12	5,11	Accidental
Cx. territans	197	4,75	15	6,38	Accidental
Cx. modestus	75	1,81	12	5,11	Accidental
Cs. annulata	20	0,48	3	1,28	Accidental
Cs. longiareolata	1665	40,18	93	39,57	Accessory
Ae. geniculatus	11	0,27	2	0,85	Accidental
Ae. vexans	7	0,17	1	0,43	Accidental
Or. pulcripalpis	20	0,48	5	2,13	Accidental
Ur. unguiculata	22	0,53	3	1,28	Accidental
Total	4144	100	235	100	
H'	1.79 bits				
H' max	3.32				
E	0.54				

FC%: relative abundance; C%: the frequency of occurrence; H': Shannon index; H' max: Maximum diversity; E: Equitability.

Table 4: Correlations Analysis (spearmen's rank) between three ecological factors (temperature, rainfall and humidity) and overall abundances of species (n = 17).

	Temperature (C°)	Rainfall (mm)	Humidity (%)	Abundance
Temperature (C°)	1	0.6732	0.00008	0.00050
Rainfall (mm)	-0.11036	1	0.52830	0.76478
Humidity (%)	-0.81154***	0.16442	1	8.38e-006
Abundance	0.75168***	-0.07843	-0.86258***	1

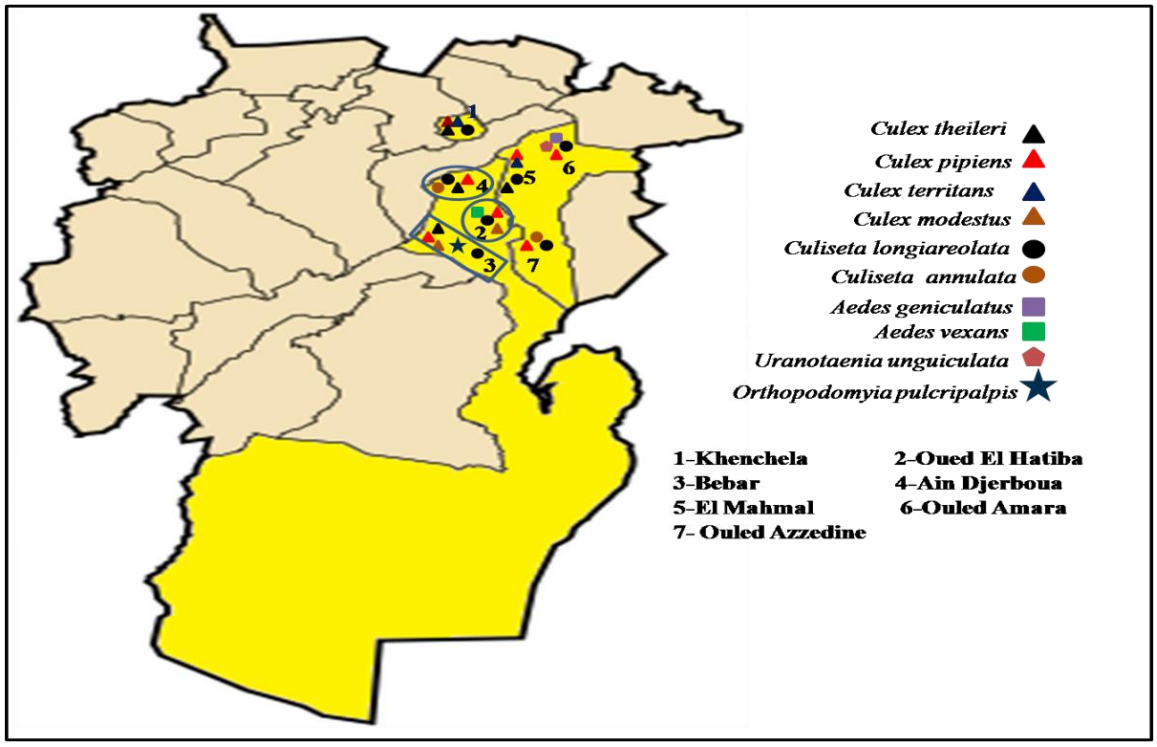

Figure 5: Distribution of the Culicidian fauna in the different sites in theKhenchela region, Algeria (from May 2020 to September 2021).

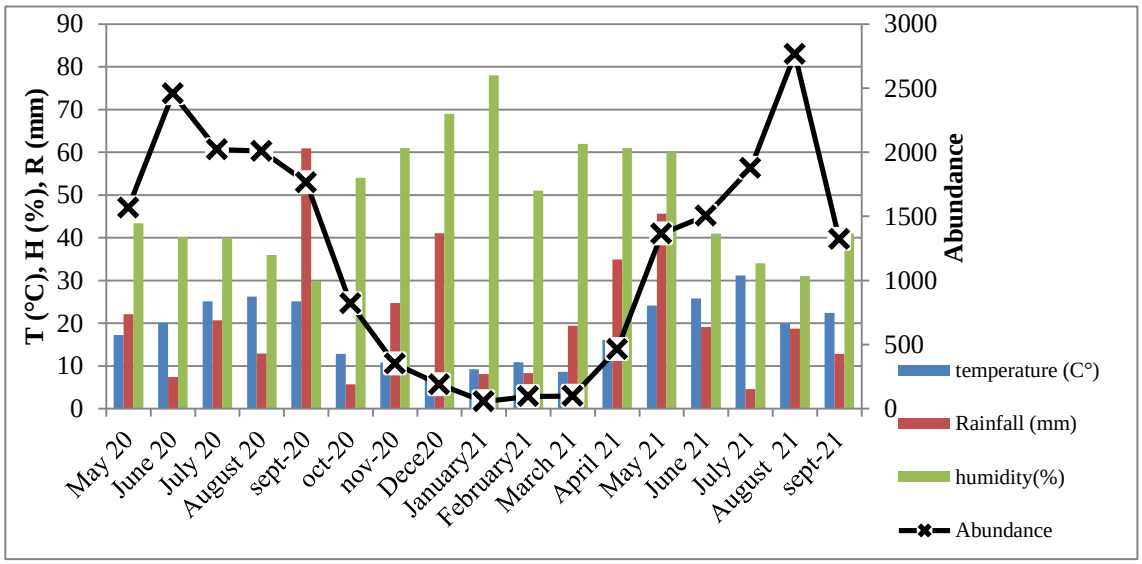

Figure 6: Monthly variations of mosquitoes overall abundances in relation with ecological factors (Temperature, Rainfall and Humidity).

DISCUSSION

The region of Khenchela had remarkable species richness with a large biodiversity of animals and plants that has not been well explored.

Our study recorded the presence of 10 species divided into 5 genera: *Culex* (*Cx. pipiens, Cx. territans, Cx. theileri*and *Cx. modestus*), *Culiseta* (*Cs. longiareolata*and *Cs. annulata*), *Aedes* (*Ae. geniculatus*and *Ae. vexans*), *Orthopodomyia* (*Or. pulcripalpis*) and *Uranotaenia* (*Ur. unguiculata*).

Senevet and Andarelli (1960), after a work of thirty years, identified 27 species in Algiers, while Brunhes et al. (2000) recorded 48 species of mosquitoes in all Algeria and Lafri et al. (2014) noted 17 species in 15 departments of Algeria. While in the Batna province (Aures region), Belkhiri et al. (2021) noted 9 species belonging to two subfamilies: Anophelinae and Culicinae.

Similar studies have been done, in Tebessa region which also has a semi- arid climate where Bouabida et al. (2012) noted 9 species in 3 genera (*Ochlerotatus*, *Culex*, *Culiseta*), while Aïssaoui and Boudjelida (2017) reported 10 species divided into 3 genera, *Culex*, *Culiseta* and *Aedes*. Similarly, in the eastern region of the country, the inventory of the culicidian fauna revealed the presence of 8, 12 and 19 species of Culicidae in Annaba (Arroussi et al., 2021), Mila (Messai et al., 2010) and Souk Ahras (Hafsi et al., 2021) respectively. Moreover, the study of Berchi (2000) conducted in the region of Constantine, reported the presence of 7 species of Culicidae belonging to 2 subfamilies (Culicinae and Anophelinae). These species are *Anopheles labranchiae, Uranotaeniaunguiculata, Culexpipiens, Cx. modestus, Cx. theileri, Cx. hortensis*and *Cs. longiareolata.*

In the Tlemcen province (Western of Algeria), 20 species of Culicidae were noted (Hassain, 2002). This relatively large number of species can be explained by the large number of samplings carried out in this region as well as the period of field survey which lasted two years. Indeed, according to Faurie et al. (1980), the number of species inventoried is in function of the number of individuals collected.Our study showed that *Culex* species were the most abundant larvae, notably *Cx. pipiens* that was collected from various breeding sites in the region. These results were consistent with those found by other authors in Algeria. For instance, in the area of Bousaada (center of Algeria), 11 species were noted and 6 belonging to genus *Culex*(Benhissen et al., 2018). For the M'sila region (south-east of Algeria), Asloum et al. (2021) revealed 14 species in total, including 10 species of the genus *Culex.* In addition, 13 species including six of*Culex*were recorded in the region of TiziOuzou (north of Algeria) (Chahed et al.,2021).

As for *Cx. pipiens*, this species was collected all year round, in different biotopes: in natural and artificial breeding sites; temporary and permanent; with and without vegetation and in clean and polluted waters, although it showed a very marked preference for deposits on polluted waters. Bueno-Marí (2020) found *Culexpipiens* the most dominant (318 individuals) among 1017 identified specimens. Besides, FilaliMouatassem et al. (2019) found from a set of 1122 larvae collected in the province of Fez (Marocco) that *Cx. pipiens* was the most abundant species (70,68 %). *Cx. pipiens* is the most abundant species in other regions of Algeria (Berchi, 2000; Benallal et al., 2015; Matoug et al., 2018; Dahchar et al., 2017; Kharoubi et al., 2020).

In terms of occurrence, *Culisetalongiareolata*and *Culexpipiens* are classified as accessory species. Hassain (2002) classified them with a very wide distribution in Mediterranean Africa. In our case, *Culisetalongiareolata* was found in 13 of 15 sites surveyed. This species existed in polluted sites, permanent sites with stagnant water with rich or poor

vegetation and in temporary water sites stagnant or common with or without vegetation (Asloum et al., 2021). Whereas *Cx.pipiens* were present in all urban sites with low frequency in rural areas. According to Berchi et al. (2012) *Cx. pipiens* frequents urban epigeal and hypogeal sites with high organic pollution and basic pH. This result shows its preference for urban anthropic sites. According to Almeida et al. (2020), anthropic environments offer favorable conditions for certain species.

Three species are classified as accidental species, which can be resulted from the environmental conditions such as water quality, the low quantity or quality of available nutrients, the drying up of the larval sites corresponding to the dry seasons, the leaching of the larval sites by rainfall and the slowing down of the larval development following the drop in temperature and mortality by invertebrate or vertebrate predators (Berchi, 2000). *Culex theileri, Cx. territans* and *Cx. modestus*are moderately represented and are present in temporary or permanent, polluted and rich vegetation sites.

The five remaining species are very rare (*Or. pulcripalpis, Ur. unguiculata, Cs. annulata, Ae. geniculatus* and *Ae. vexans*). *Aedes geniculatus* and *Uranotaenia unguiculata* were found only in the site Ouled Amara, knowing that the first species does not play any role in the transmission of human parasitosis (Brunhes et al., 1999) and it was found previously in TiziOuzou in northern Algeria (Chahed et al., 2021). However, *Ae. vexans* has been inventoried in only one site in our investigation (Oued Elhatiba) and it was also identified also in the province of Biskra (southeast of Algeria) (Benhissen et al., 2014).

Over the past two decades, several studies in Algeria have been dealing with mosquito. Considering the fact that some vector mosquitoes of important diseases, such as West Nile Virus, Filariasis and Malaria were recorded in the several regions (Arroussi et al.,2021),the studies on the composition and distribution of mosquito species is very important to elaborate vector control programs and monitoring strategies.

The present study showed that most identified species are potential vectors of pathogens. For instance, *Cx. pipiens* ,*Cx. modestus* and *Cx. theileri*are known as a potential vectors for West Nile Virus disease (Brunhes et al., 1999 ; Amraoui et al., 2012; Golding et al., 2012). Whereas, *Ae. vexans* play a role in the transmissionof Rift Valley Fever virus (Birnberg et al., 2019). Besides, *Ur.unguiculata* is a vector species of parasites of amphibians and reptiles (Brunhes et al., 1999) and it was found in the region of Skikda (Matoug et al., 2018) and *Cs. longiareolata* is a vector of avian Malaria, Tularemia, Malta Fever and arbovirus such as West Nile Fever (Khaligh et al., 2020).

Thus, the obtained results may contribute to the development of an efficient entomological monitoring and vector control strategy in the studied region.

On the other hand, the diversity of species is more significant in a given habitat when the living conditions are favorable. The calculated value of the Shannon Weaver index at the level of the sites surveyed in the region of Khenchela is 1.79 bits, this allows us to conclude that the culicidian population is moderately diversified. A close diversity index (1.85 bits) was found by Chahed et al. (2021) in the region of TiziOuzou, while Hamaidia and Berchi (2018) found a higher value of 2.50 bits in the same region which explains a medium diversity environment. The equitability value is equal to 54 %, it shows that the species found do not have the same abundances (average balance between culicid species collected) in the region of Khenchela. The scarcity of some species can be explained by the number of field samplings made in each station, the durability and location of the sites, the drying up of the sites during the drought

period and the climatic conditions. Furthermore, we found that climatic factors (especially temperature and humidity) and species abundances are correlated. Hence, climate change affects the spread of many species, which disrupts their ability to adapt, resulting in a significant loss of biodiversity (Merabti and Ouakid, 2011).

The climatic variations during the year played a great role in the abundance of mosquitoes collected during our field trips. Our results show that the presence of mosquitoes is not the same during the whole study period, and that they were present in variable numbers depending on three ecological factors (temperature, humidity and rainfall). The increase in the number of individuals in the first peak which was between May and June is reflected by the increase of aquatic habitats that are suitable for mosquito breeding in the visited sites as well as the rainfall for the months preceding these peaks. On the other hand, during the winter (cold season), the abundance of mosquitoes was reduced due to the destruction of some breeding sites by rainfall or the instability of others.

CONCLUSION

This work shows that the region of Khenchela (Northeast Algeria) harbor some mosquito species of medical importance, which is a complement to other mosquito surveys performed, adds to our knowledge of the distribution of medically important species in Algeria. Among the 10 species of Culicidae found, most of the species identified are potential vectors of vector-borne diseases (in humans or animals), namely: *Cx. pipiens*, *Cx. theileri*and *Cx. modestus* which are vectors of West Nile virus fever and *Ae. vexans* which is involved in the transmission of Rift Valley fever virus. These results are useful for an effective control program, by focusing on the most abundant and dangerous species, and choosing the appropriate tools to limit their populations. This study, although preliminary, deserves to be further expanded by inventories in other regions of the province, with a longer study period and deepened by proteomic and genomic approaches in order to establish a phylogenetic tree, which could elucidate some systematic problem.

CONFLICT OF INTEREST

The authors declar that they have no conflict of interest.

AUTHORS CONTRIBUTION

IH: Mosquito collections, performed the experiments,analyzed data and wrote the paper, FZKT: conceived and supervised study, LS: Contibuted in analysis of the data and drafted the article, MRB: Validation of identification and revised manuscript, AG and FS: participed in the study design, read manuscript and revised it.

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