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FACULTY OF SCIENCES

BIOLOGY DEPARTMENT

LABORATORY OF MARINE AND COASTAL ECOBILOGY

Thesis for the award of a Doctorate Degree

Field: NATURAL AND LIFE SCIENCE

Branch: ECOLOGY AND ENVIRONMENT

Specialty: ECO-ETHOLOGY

**Entitled**

# « Toxic effects of secondary compounds of spontaneous plants on some Culicidae species».

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**Thesis supervisor :** Dr. Yasmine Adjami

**Thesis Co-supervisor :** Pr. Mohamed Laid OUAKID

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UNIVERSITÉ BADJI MOKHTAR –ANNABA

جامعة باجي مختار - عنابة

FACULTÉ DES SCIENCES

DÉPARTEMENT DE BIOLOGIE

LABORATOIRE D'ECOBILOGIE DES MILIEUX MARINS ET LITTORAUX

Thèse En vue de l'obtention d'un Diplôme de Doctorat

Domaine : SCIENCE DE LA NATURE ET DE LA VIE
Filière : ECOLOGIE ET ENVIRONNEMENT
Spécialité : ECO-ETHOLOGIE

**Intitulé**

# « Effets toxiques des composés secondaires des plantes spontanées sur quelques espèces de Culicidae».

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# *Dedication*

*I dedicate this work to two people very dear to my heart, those who sacrificed their lives for mine. Let this work be a testimony of my gratitude and let these words be an affirmation of my deepest love.*

*To my mother, an exceptional woman who gave me a wonderful model of hard work, patience and perseverance.*

*To my father, I give you this work as the fruit of your sacrifices so that we can live better and I will never forget your advices because it pushed me to get here.*

*Thank you for making me want to go further. May Allah the Almighty preserve you and grant you health, happiness and long life!*

*I thank my husband, my two dear brothers, my grandparents, and all my family.*

*I love You dearly.*

## Abstract:

Mosquitoes are both useful and dangerous species but their ability to be a public health threat has become a priority due to the increase of death numbers by arboviruses. Our work was conducted to both know and understand these species and also to try control methods and substance of them. Culicidae inventory in the region of Annaba El Tarf and Guelma showed the presence of 9 species *Aedes caspius*, *Aedes geniculatus*, *Aedes albopictus*, *Culex pipiens*, *Culex hortensis*, *Culex theileri*, *Culex perexiguus*. *Culiseta longiareolata*, and *Anopheles labranchiae*. As the most abundant species is *Culex pipiens*. Thus, the ovitraps showed interesting results concerning *Aedes albopictus* that came recently to our country but just in a few years, it shown to be very adaptive and nondemanding when it comes to its preferences in breeding sites. After revealing the arrival of the Asian tiger mosquito to the region of Annaba in 2017, we also declared it for the first time in both Bouchegouf (Guelma) and Besbes (El Tarf), to confirm that most of Northeast Algeria is infested with *Aedes albopictus*. We determined that this species prefers urban artificial breeding sites. In Besbes (El Tarf), the ovitraps revealed *Aedes albopictus* dominance over native species *Culex pipiens* and *Culiseta longiareolata* (82,09 %) ($F_{2,69}$= 7,14; P= 0,002). Thus, this invasive and adaptive species could coexist with local mosquito species, it can even displace them. The toxicity test using Spinosad on *Culex pipiens* larvae got us an idea of the Lc50 and LC90 which are 119.47 and 497.3 µg / l successfully. Showing both efficiency and no threat to ecosystems.

**Key words:** North-east Algeria, Culicidae, *Aedes albopictus*, toxicity, *Culex pipiens*, Spinosad.

## Résume :

Les moustiques sont à la fois des espèces utiles et dangereuses, mais leur capacité à constituer une menace pour la santé publique est devenue une inquiétude en raison de l'augmentation du nombre de décès par les arbovirus transférai par ces vecteurs. Notre travail a été mené à la fois pour connaître et comprendre ces espèces et aussi pour avoir des méthodes de contrôle utile. L'inventaire des Culicidae dans la région d'Annaba El Tarf et Guelma a montré la présence de 9 espèces *Aedes caspius*, *Aedes geniculatus*, *Aedes albopictus*, *Culex pipiens*, *Culex hortensis*, *Culex theileri*, *Culex perexiguus*. *Culiseta longiareolata* et *Anopheles labranchiae*. Comme l'espèce la plus répandue est *Culex pipiens* alors que les ovitraps ont montré des résultats intéressants concernant *Aedes albopictus* arrivé récemment dans notre pays mais en quelques années, elle s'est montrée très adaptative et peu exigeante en ce qui concerne ses préférences en matière de sites de reproduction. Après avoir révélé l'arrivée du moustique tigre asiatique dans la région d'Annaba en 2017, nous l'avons également déclaré pour la première fois à Bouchegouf (Guelma) et à Besbes (El Tarf), pour confirmer que la majeure partie du Nord-Est algérien est infestée par *Aedes albopictus*. Nous avons déterminé que cette espèce préfère les sites de reproduction artificiels urbains. A Besbes (El Tarf), les ovitraps ont révélé une dominance d'*Aedes albopictus* sur les espèces indigènes *Culex pipiens* et *Culiseta longiareolata* (82,09 %) ($F_{2,69}$= 7,14 ; P= 0,002). Ainsi, cette espèce envahissante et adaptative pourrait coexister avec des espèces de moustiques locales, elle peut même les déplacer. Le test de toxicité à l'aide de Spinosad sur des larves de *Culex pipiens* nous a donné une idée sur les LC50 et LC90 qui sont de 119,47 et 497,3 µg/l avec succès. Démontrant à la fois efficacité et absence de menace pour les écosystèmes.

**Mots clés :** Nord-Est algérien, Culicidae, *Aedes albopictus*, toxicité, *Culex pipiens*, Spinosad.

## الملخص:

يعتبر البعوض من الأنواع المفيدة والخطيرة على حد سواء، لكن قدرته على تشكيل تهديد للصحة العامة أصبحت مصدر قلق حيث كانت سببا في زيادة عدد الوفيات الناجمة عن الفيروسات التي تنقلها. أنجز هذا العمل لمعرفة وفهم هذه الأنواع من الحشرات والقيام بعمليات حصر من أجل وضع طرق مفيدة للتحكم. أظهرت عمليات حصر البعوض في منطقة عنابة والطارف وقالمة وجود 9 أنواع *Aedes caspius* و *Aedes geniculatus* و *Aedes albopictus* و *Culex pipiens* و *Culex hortensis* و *Culex theileri* و *Culiseta longiareolata*. *Culex perexiguus* و *Anopheles labranchiae*. بينما كانت *Culex pipiens* النوع الأكثر انتشارا إلا أن مصائد اليرقات بينت نتائج مثيرة للاهتمام تتعلق بـ *Aedes albopictus* التي انتشرت بشكل رهيب في بلادنا في غضون بضع سنوات، وأثبتت أنها شديدة التكيف مع الوسط ومتساهلة في شروط اختيار مواقع التكاثر. بعد اكتشاف وصول بعوضة النمر الآسيوية إلى منطقة عنابة في عام 2017، أعلنا من خلال هذا العمل ولأول مرة ظهورها في بوشقوف (منطقة قالمة) جنوب عنابة وفي البسباس (منطقة الطارف) في شرقها، وهو ما يؤكد انتشارها الواسع في شمال شرق الجزائر. كما أكدنا تفضيل هذا النوع لمواقع التكاثر الاصطناعية في المناطق الحضرية. وبينت مصائد البيض في البسباس (منطقة الطارف) سيادة *Aedes albopictus* على النوعين المحليين *Culex pipiens* و *Culiseta longiareolata* بنسبة (% 82,09) ٪82.09 (7.14 = F2.69 ؛ P = 0.002). وهو ما يؤكد ليس فقط تعايش هذا النوع مع الأنواع المحلية بل يمكنه أن يحل محلها. أعطى اختبار السمية باستخدام مبيد Spinosad على يرقات *Culex pipiens* فكرة عن LC50 و LC90 بقيمة 119.47 و 497.3 ميكروغرام / لتر. وأظهر فعالية مع خصائصه المعروفة بعدم تهديده للنظم البيئية.



**الكلمات المفتاحية:** شمال شرق الجزائر، البعوضيات، *Aedes albopictus*، السمية، *Culex pipiens*، Spinosad

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# Abbreviations

***Ae***.: *Aedes*

***An***.: *Anopheles*

**ANOVA**: Analysis of Variance

**C (%)**: Constancy or index of occurrence

**C**: Concentration index

**CF**: Centesimal frequency

**CM**: corrected mortality

***Cs***.: *Culiseta*

***Cx***.: *Culex*

**E**: Equitability index

**GABAergic**: synapse of Gamma-Aminobutyric Acid

H**:** Humidity

**H'**: Shannon & Weaver diversity index

**$I_a$**: Aridity index

**$L_4$**: the 4th stage of larvae

**LC50**: lethal concentration of 50% of the treated

**LC90**: lethal concentration of 90% of the treated

**nAChRs**: Nicotinic acetylcholine receptors

**OM**: observed mortality

**P**: Precipitation

**Q3**: Rainfall Quotient of Emberger

**RA**: Relative abundance

**S**: Total species richness

**Sm**: Richness mean

**T**: Annual temperature

**WHO**: World Health Organization

**ZIKAV**: Zika virus

# Summary

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# Introduction

Mosquitoes are the most important hematophagous Diptera in public health and a major vector for the most important and dangerous infections to humans, such as malaria, filariasis, Japanese encephalitis, dengue, yellow fever, etc. **(Ghosh *et al.*, 2012; Kamatchi *et al.*, 2016)**.

The distribution of vector-borne diseases is determined by complex dynamics involving both environmental and social factors. The abundance of standing water, urbanization, the development of irrigated agriculture, climate change, and the globalization of travel and trade are all factors that can cause epidemics in countries that have not experienced and promote the emergence of vector-borne diseases such as: chikungunya, a major global public health event, is perhaps, along with dengue, one of the best illustrations of these emergence factors **(Fischer *et al*., 2013; Campbell *et al*., 2015; Medlock *et al*., 2015)**. The spread of these diseases is closely linked to the geographical expansion of the main vector species, Aedes aegypti and Ae. albopictus **(Higgs & Vanlandingham, 2015).**

The worldwide spread of *A. albopictus* began with the Pacific and Indian islands, followed by North America in 1985, Brazil and other South American nations in subsequent years. *Ae. albopictus* only reached Europe in 1979, arriving in Albania presumably as eggs or larvae in used water-filled tires **(Adhami *et al.*, 1998).** It has now expanded through the Peri-Mediterranean area **(Kraemer *et al.*,2015)**, east to Georgia **(Kutateladze *et al.*, 2016)**, and west to the remaining parts of southern Europe. **(Marabuto & Rebelo, 2018).**

The first recorded sighting of *Ae. albopictus* on the African continent was reported in 1989 in the Republic of South Africa **(Cornel & Hunt, 1991)**. The following year, the distribution of the Asian Tiger mosquito extended to Nigeria, according to **Savage *et al.* (1992)**, and thereafter it increased rapidly to the coastal regions of Western and Central Africa, confirmed by **Fontenille & Toto, (2001); Toto *et al*. (2003)**.

Given Algeria's geographical location, its climate, and the increasing development of international trade, particularly with Europe and other African countries, the study of the Culicidae fauna of Algeria is the subject of a large number of studies that focus on the systematics, biochemistry, morphometrics, chemical, and biological control of mosquitoes in different regions of the country. The incidental presence of *Aedes albopictus* was first reported in Algeria in June 2010 in Larbaa-Nath-Iraten (Wilaya of Tizi-Ouzou) **(Izri *et al.*,**

**2011)**, in December 2015 in Ain Turk (Wilaya of Oran) **(Benallal *et al*., 2016)**, and in July 2016 between Birkhadem and Ain Naadja (Wilaya d'Alger) **(Benallal *et al*., 2019)**, Annaba in 2018 **(Arroussi *et al*., 2021),** and Souk Ahras in the summer of 2020 **(Hamaidia & Soltani, 2021),** and we plan to record this species in the region of El Tarf and Guelma in 2021 **(Rouibi *et al*., 2023).**

Chemical control, mainly with synthetic chemical pesticides, continues to be the major means of vector control **(Cassida & Quistad, 1998)**. However, the use of conventional insecticides has had long-term side-effects including the emergence of resistant species **(Boyer, 2006; Toma *et al*., 2011)**. Environmental imperatives **(Paoletti & Pimentel, 2000)** have encouraged the search for emergency measures, based on the elaboration of adequate strategies in the use of pesticides and the development of new selective molecules with low eco-toxicological risks **(Dhadialla *et al*., 2010; Hui *et al*., 2013)**.

Spinosad (SP) is a bioinsecticide product obtained from the fermentation of actinomycete *Saccharopolyspora spinosa* **(West., 1997)** that acts primarily by disrupting nicotinic acetylcholine receptors **(Kirst., 2010)**. It is considered an environmentally safe insecticide and constitutes an alternative to broad spectrum insecticides due to the low toxicity in mammals and the low doses required **(Zhao *et al*., 2007)**. Nonetheless, these and many other bioinsecticides are photodegradable and are rapidly affected by biodegradation **(Varikou *et al*., 2018; Cleveland *et al*., 2002)**.

This work is a contribution to a behavioral and toxicological study on some species of mosquitoes to get an idea of their resistance capacity. We started this work by making an inventory in the region of North East Algeria in the Wilaya of Annaba El Tarf and Guelma to get an idea of the population of Culicidae in this region and their distribution by calculating ecological indices.

The second part is devoted to a study of the oviposition of *Aedes albopictus*. We installed Ovitraps to collect larvae, identify them, and know the life cycle of this species and its relation with other species in the north-east of Algeria (Annaba El Tarf and Guelma).

Last but not least, toxicological tests were carried out on *Culex pipiens* larvae using Spinosad, a pesticide of natural origin that was claimed to be ecofriendly.

# I. Material & methods

## 1. Description of the study area:

The study area is located on the southern shore of the Mediterranean basin, in the northeast of Algeria. Annaba and El Tarf are two coastal plain regions that have industrial and agricultural activities with economic and fishing harbors, while Guelma is an inland mountain region with agricultural activities. They enjoy a Mediterranean climate where precipitation is abundant and can be torrential, and it’s generally hot, especially from June to August.

Mosquitoes have an aquatic larval stage, so the diversity of aquatic sites between natural and artificial has been taken into consideration, and the choice of sites was made according to the impact of the climatic and geographical diversity of the sites studied.

### 1.1. The geographic location:

Annaba is located 600 km from the capital Algiers and 100 km from the Tunisian border. The city rises at the bottom of a bay open to the east by the Mediterranean gulf. It is dominated to the west by the Edough mountain range (1008m above sea level).

El Tarf is located in the extreme north-east of Algeria with 2908 $km^2$ of area to the east of the Algerian-Tunisian border, to the west the wilaya of Annaba, like the latter El Tarf is a coastal wilaya, to the east and south are the wilayas of Skikda, Guelma and Souk Ahras.

Guelma occupy a major agricultural region, 290 m above sea level and surrounded by mountains (Mahouna, Dbegh, Houara and Taya (Bouhamdane)). The region is very fertile because of the Seybouse River and a large dam that provides a vast irrigation scheme. It occupies a strategic geographic position as a crossroads in north-eastern Algeria, linking the coast of Wilaya of Annaba, El Tarf and Skikda in the north to inland areas such as Wilaya of Constantine in the west, Souk-Ahras in the east, and Oum El Bouagui in the south **(Figure 01)**.


**Figure 01.** Position and geographical location of the study area.

### 1.2. Hydrography

As we know that the most important element for mosquitos' survival is water, we mansion the most important water bodies in the wilayas studied **(Figure 02)**.

#### 1.2.1. Guelma:

- **Main Oueds :**

**- Oued Seybouse**: it has its source at Medjez Amar (meeting point of Oued Charef and Oued Bouhamdane). It crosses Guelma and Bouchegouf plain over more than 45 km from south to north. Its total contribution is estimated at 408 million $m^3$/year at the Bouderoua station (Ain Ben Beida commune).

**- Oued Bouhamdane**: which has its source in the Commune of Bouhamdane in the west of the Wilaya. Its contribution is 96 million m3/year to the Medjez Amar II station.

**- Oued Mellah**: coming from the South-East, this watercourse records a total contribution of 151 million m3/year to the Bouchegouf station.

**- Oued Charef**: Takes its source in the South of the Wilaya and its contribution is estimated at 107 million m3/year at the Medjez Amar I station.

- **Existing dams:**

Hammam Debagh dam on Oued Bouhamdane with a capacity of 220 $HM^3$.

Medjez Beggar dam (Ain-Makhlouf) with a capacity of 2,786 $HM^3$.

**1.2.2. Annaba:**

- **Oued Seybouse:**

It has its mouth at the south-east of the city which joins the Mediterranean Sea. Its basin is the largest in Algeria and its land is the most fertile.

- **Fetzara lake:**

It is located west of the city, 14 km from the Mediterranean Sea. It extends in an east-west direction over 17 km in length and 13 km in width. It is limited to the north by the Edough massif, by the hills of Aïn Berda to the south and by the dune ridges to the east and west. On the periphery of the lake there are several settlements.

The maximum storage capacity of the lake basin is estimated at about 84 million cubic meters corresponding to a water height of 2.75 meters **(Mouhoub, 2012)**. The open water body, whose fresh water is relatively temporary depending on the intensity of the rainy season, is generally over 5,800 hectares in size.

**1.2.3. El Tarf:**

The dune zone, with a surface area of around 12,000 ha, i.e. 04% of the ST. The El Kala National Park, which represents almost a third (1/3) of the total surface area (800 $km^2$), 12% of which is classified as "Integrated Reserves". This wilaya is rich in wetlands.

- **Wetlands:**

The stagnant waters, which play a predominant role in the hydrology and hydrogeology of the region, form numerous marshes and lakes which are the marshes of M'Khada, bird's lake, and the blue lake, classified in the Ramsar list of protected areas.

The whole area is classified as a "Biosphere Reserve" in the framework of protected species. The Tonga and Oubeira lakes are protected by the RAMSAR convention on wetlands of international importance

Besides these large lakes, there are others with unique biological and geological characteristics such as Lac Noir el Mellah, El Betah, and many underrated wetlands.

- **Dams and oueds:**

La wilaya d'El Tarf dispose de trois barrages opérationnels (Meksa, Bougous et Chaffia) d'une capacité de stockage de 252 millions de mètres cubes.

The Cheffia dam is located in the middle of a forest region. It is located on the banks of the oued Bounamoussa. The latter has an estimated terrain elevation above sea level of 4 meters. The volume of water of the Chafia dam (El Tarf), the main hydraulic work that supplies the wilayas of Annaba and El Tarf, was increased to 38 million $m^3$.

The Meksa dam received 20 million $m^3$ with a filling rate of 85% and together with the Bougous dam these two feed El Oued El-Kbir. These two wadis (Bounamoussa and El-Kbir) meet at a point called El Mafrague.

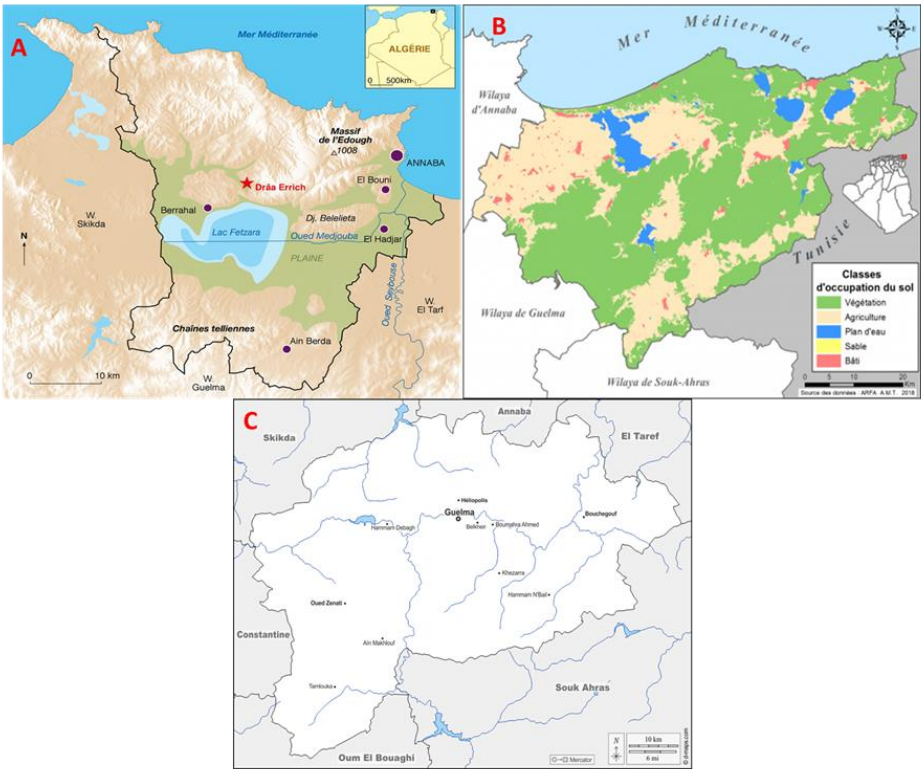


**Figure 02.** The distribution of water bodies in the study area (A : Annaba, B : El Tarf et C : Guelma).

### 1.3. Climatic data from different surveyed regions:

Climate is the result of different elements, such as temperature, precipitation, wind and relative humidity, which can directly or indirectly affect living beings. The climate data has been retrieved from the website **fr.climate-data.org**.

#### 1.3.1. Climatic data for Annaba region:

**Table 01:** Climatic data for the Annaba region for the period 1991 to 2021 **(fr.climate-data.org)**

*[**Tm:** average of the minimum temperatures of the month (°C); **TM**: average of the maximum temperatures of the month (°C);*

***T = (m+M/2):** average temperature (°C)**,V:** wind (Km/h), **P**: precipitation**, H:** humidity**]***

| Month | TM C° | Tm C° | T C° | V(Km/h) | P (mm) | H(%) |
|---|---|---|---|---|---|---|
| **January** | 14,1 | 6.6 | 10.2 | 17.8 | 95 | 77 |
| **February** | 14,4 | 6.5 | 10.3 | 17.6 | 80 | 76 |
| **March** | 17.3 | 8.2 | 12.6 | 16.3 | 68 | 75 |
| **April** | 20.1 | 10.6 | 15.3 | 15.3 | 61 | 73 |
| **May** | 24 | 14 | 18.8 | 13.3 | 40 | 68 |
| **June** | 29 | 18 | 23.4 | 11.9 | 11 | 59 |
| **July** | 32.5 | 21.1 | 26.6 | 11.8 | 2 | 55 |
| **August** | 32.6 | 21.8 | 26.8 | 11.6 | 12 | 57 |
| **September** | 28.5 | 19.5 | 23.6 | 12.5 | 43 | 65 |
| **October** | 25.2 | 16.2 | 20.3 | 13.9 | 64 | 69 |
| **November** | 18.9 | 11.2 | 14.8 | 16.5 | 97 | 73 |
| **December** | 15.3 | 8 | 11.5 | 17.9 | 98 | 76 |
| **Mean / an** | **22.66** | **13.48** | **17.85** | **14.7** | **55.92** | |
| **Total** | | | | | **671** | |

❖ **Temperature:**

Temperature is a very important limiting factor, as it controls all metabolic phenomena and thus conditions the distribution of all species and communities of living beings in the biosphere **(Ramade 1984).**

The results recorded in the Wilaya of Annaba show that the Annaba region is characterized by temperatures averaging around **(17.85 C°).** January has the lowest average temperature (**10.2 C°**). The highest temperatures are recorded in August with an average of 26.8 C° **(Table 01).**

❖ **Precipitation:**

Over the 30 years (1991/2021) Annaba receives an average of 55.92 mm per month and an annual total of 671 mm, the month of December is considered to be the wettest month with 98 mm, in contrast to the month of July during which the smallest amount of rainfall was recorded which is 2 mm **(Table 01).**

❖ **Wind:**

Wind is an important agent of erosion and therefore of desertification, it increases evapotranspiration and contributes to drying out the atmosphere **(Mackenzie *et al*., 2000).** In the Annaba region, winds are relatively frequent throughout the year. The maximum wind speed was 17.9 km/h in December and the minimum value was recorded in October with 11.6 km/h for an average of the years (2015-2023) **(Table 01).**

❖ **Humidity:**

The relative humidity of the air varies significantly with the seasons. In summer, it drops to 55% in July due to high evaporation. On the other hand, in winter, it rises to 77% (maximum value) in January. **(Table 01)**

### 1.3.2. Climatic data of El Tarf region:

**Table 02 :** Climatic data for the region of El Tarf for the period 1991 to 2021 **(fr.climate-data.org)**

*[**Tm:** average of the minimum temperatures of the month (°C); **TM**: average of the maximum temperatures of the month (°C);*

***T = (m+M/2):** average temperature (°C)**,V:** wind (Km/h), **P**: precipitation**, H:** humidity**]***

| **Month** | **TM C°** | **Tm C°** | **T C°** | **V(Km/h)** | **P (mm)** | **H (%)** |
|---|---|---|---|---|---|---|
| January | 14.3 | 8.4 | 11.2 | 16.9 | 117 | 76 |
| February | 14.4 | 8 | 11.1 | 16.9 | 101 | 75 |
| March | 16.8 | 9.7 | 13.2 | 16 | 89 | 75 |
| April | 19.2 | 11.7 | 15.4 | 15.1 | 79 | 75 |
| May | 22.4 | 14.5 | 18.5 | 13.3 | 54 | 74 |
| June | 26.7 | 18 | 22.5 | 12 | 17 | 67 |
| July | 30 | 20.9 | 25.6 | 11.9 | 3 | 63 |
| August | 30.6 | 21.8 | 26.2 | 11.4 | 15 | 63 |
| September | 27.4 | 19.9 | 23.5 | 12.1 | 53 | 69 |
| October | 24.4 | 17.2 | 20.6 | 13.3 | 78 | 71 |
| November | 19 | 12.9 | 15.7 | 15.7 | 114 | 73 |
| December | 15.5 | 9.8 | 12.5 | 16.9 | 114 | 75 |
| **Mean / an** | **21.73** | **14.4** | **18** | **14.29** | **69.5** | |
| **Total** | | | | | **834** | |

- **Temperature :**

Temperature is a good indicator of the climate of the regions. The results recorded in the wilaya of El Tarf show that the average annual temperature for the years (1991-2021) is **18°C** with strong seasonal variations (26.6°C in August and 11.1°C in February). **(Table 02)**

- **Precipitation:**

Over the 30 years (1991/2021) the wilaya of El Tarf receives an average of 69.5 mm per month and an annual total of 834 mm, the month of January is considered the wettest month with 117 mm, contrary to the month of July during which it recorded the smallest amount of rain which is 3 mm **(Table 02).**

- **Wind:**

In the region of El Tarf, winds are relatively frequent throughout the year. The maximum wind speed was 16.9 km/h in January and the minimum value was recorded in August with 11.4 km/h for an average of 10 years (2015-2023) **(Table 02).**

- **Humidity:**

The relative humidity of the air varies significantly with the seasons. In summer, it drops to 63% in August and July due to high evaporation. On the other hand, in winter, it rises to 76% (maximum value) in January **(Table 02).**

### 1.3.3. Climatic data of The region of Guelma:

**Table 03 :** Climate data for the region of Guelma from 1991 to 2021 **(fr.climate-data.org)**

*[**Tm:** average of the minimum temperatures of the month (°C); **TM**: average of the maximum temperatures of the month (°C);*

***T = (m+M/2):** average temperature (°C)**,V:** wind (Km/h), **P**: precipitation**, H:** humidity**]***

| **Month** | **TM C°** | **Tm C°** | **T C°** | **V(Km/h)** | **P (mm)** | **H(%)** |
|---|---|---|---|---|---|---|
| January | 13.1 | 3 | 7.7 | 14.1 | 72 | 77 |
| February | 13.5 | 3 | 8 | 14.1 | 64 | 76 |
| March | 17 | 5.4 | 10.9 | 13.7 | 75 | 73 |
| April | 20.2 | 7.9 | 13.9 | 13.3 | 71 | 71 |
| May | 24.4 | 11.3 | 17.7 | 11.9 | 59 | 66 |
| June | 30 | 15.3 | 22.7 | 11.2 | 20 | 53 |
| July | 34.2 | 18.5 | 26.4 | 11.1 | 5 | 45 |
| August | 34 | 19 | 26.2 | 10.8 | 20 | 47 |
| September | 28.8 | 1.5 | 22.2 | 10.9 | 42 | 59 |
| October | 24.7 | 13 | 18.4 | 11.8 | 62 | 65 |
| November | 18 | 7.8 | 12.5 | 13.4 | 68 | 72 |
| December | 14.2 | 4.4 | 8.9 | 14.2 | 65 | 76 |
| **Mean / an** | **22.68** | **10.43** | **16.29** | **12.54** | **51.92** | |
| **Total** | | | | | **623** | |

- **Temperature :**

Temperature is a good indicator of the climate of the regions. The results recorded in the Wilaya of Guelma show that the average annual temperature for the years (1991-2021) is 19.7°C with strong seasonal variations (26.4°C in July and 7.7°C in January) **(Table 03).**

- **Precipitations :**

Over the 30 years (1991/2021) the Wilaya of Guelma receives an average of 51.92 mm per month and an annual total of 623 mm, the month of January is considered to be the wettest month with 72 mm, in contrast to the month of July during which the smallest amount of rainfall was recorded which was 5 mm **(Table 03).**

- **Wind :**

In the region of Guelma, winds are relatively frequent throughout the year. The maximum wind speed was 14.2 km/h in December and the minimum value was recorded in August at 10.8 km/h for an average of 9 years (2015-2023) **(Table 03).**

- **Humidity :**

The relative humidity of the air varies significantly with the seasons. In summer, it drops to 45% in July due to high evaporation. On the other hand, in winter, it rises to 77% (maximum value) in January **(Table 03).**

**1.3.4. Climatic analysis:**

In order to characterize the climate of our study area, we determined the aridity index, the Gaussian ombrothermic diagram and the Emberger climagram.

- **Gaussian ombrothermic diagram:**

The ombrothermal diagram of **(Bagnouls & Gaussen, 1953)** is a graphic method that allows us to determine the dry period. A month is considered to be biologically dry when $p \leq T$, with P: average rainfall in mm and T: the average temperature in °C.

The analysis of the diagram **(Figure 03)** shows that the climate of the three regions studied (El Tarf, Annaba and Guelma) is marked by a dry period of four months from June to September.

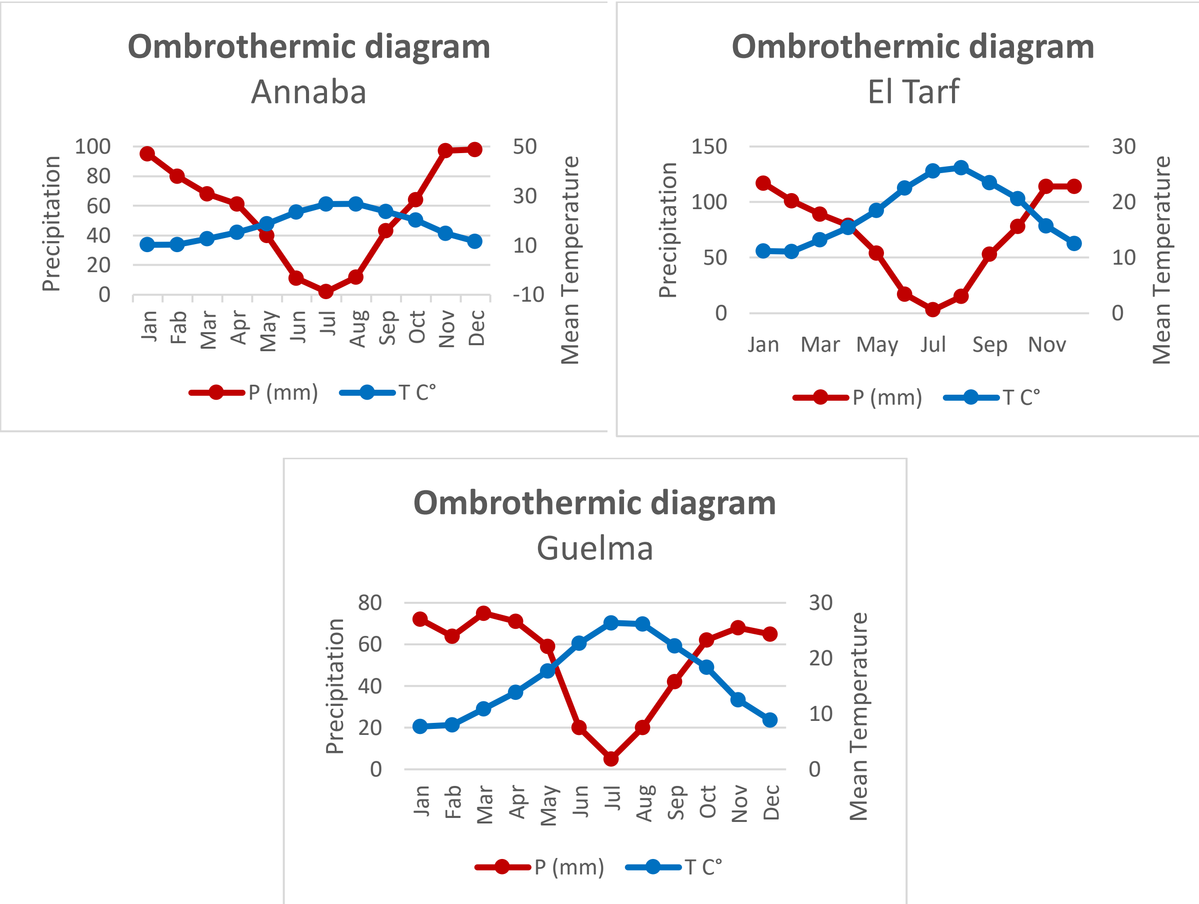


**Figure 03:** Gaussian embrothermal diagram of the wilayas: Annaba, El Tarf and Guelma (1991-2021).

❖ **Martonne aridity index 1925 :**

The formula is given as follows: $$I_a = \frac{P}{T+10}$$

P : annual pluviometry in (mm).
T : average annual temperature in (C°).
10 : coefficient added so as not to have a negative index.

The regions are classified according to the following limits **(Table 04)**:

**Table 04.** De Martonne Aridity Index Ranking.

| Values of Ia | Type of climate |
|---|---|
| **$I_a \leq 5$** | Hyper arid climate |
| **$5 \leq I_a \leq 7.5$** | Desertic climate |
| **$7.5 \leq I_a \leq 10$** | Steppe climate |
| **$10 \leq I_a \leq 20$** | Semi-arid climate |
| **$20 \leq I_a \leq 30$** | Temperate climate |
| **$30 \leq I_a$** | Humid climate |

From the climatic data for the period (1991/2021) of the region of Annaba, Guelma the aridity index "Ia" is **24.09** and **23.70** successively which indicates that the two regions are characterized by a semi-arid climate. On the other hand, the aridity index of El Tarf is **29.79**, which indicates that this region has a sub-humid climate.

❖ **Emberger's Climagram:**

The EMBERGER climagram is used to determine the bioclimatic stage of a given station. It is determined from the following formula developed by **(Stewart, 1969)** indicates the rainfall quotient (Q3) which he proposed by the following formula:

$$Q_3 = 3.43\ (P/M\text{-}m)$$

P : annual rainfall (mm).
M : maximum average of the warmest month (C°).
m : minimum average of the coldest month (C°).

**Stewart (1969),** therefore, simplified the EMBERER formula by calculating a Q3 for North African stations. This is a mathematical improvement of Q2 where the temperatures M and m are expressed in °C. EMBERGER considered that Q2 was not sufficient to characterize a station and that it was essential to include the average minimum temperature of the coldest month (m), which plays an essential role in the start of vegetation growth. EMBERGER constructed a climagram with Q2 on the ordinate and the average minimum temperature of the coldest month (m) on the abscissa, on which the different bioclimatic stages (Saharan, arid, semi-arid, subhumid, humid, and per-humid) are plotted. Each bioclimatic stage has a corresponding thermal variance or sub-stage: **(Bekkar, 2012)**

m< 0 : Cold winter
0 ≤ m <3 : Cool winter
3 ≤ m < 7 : Warm winter
m ≥ 7 : Hot winter

**Table 05.** Rainfall quotient and the different floor of each study area.

| **Regions** | **M** | **m** | **P** | **$Q_2$** | **Bioclimatic floor** |
|---|---|---|---|---|---|
| Annaba | 26.8 | 10.2 | 671 | 138.64 | Subhumid with warm winter |
| El Tarf | 26.6 | 11.1 | 834 | 184.56 | Humid with warm winter |
| Guelma | 26.4 | 7.7 | 623 | 114.27 | Subhumid with warm winter |

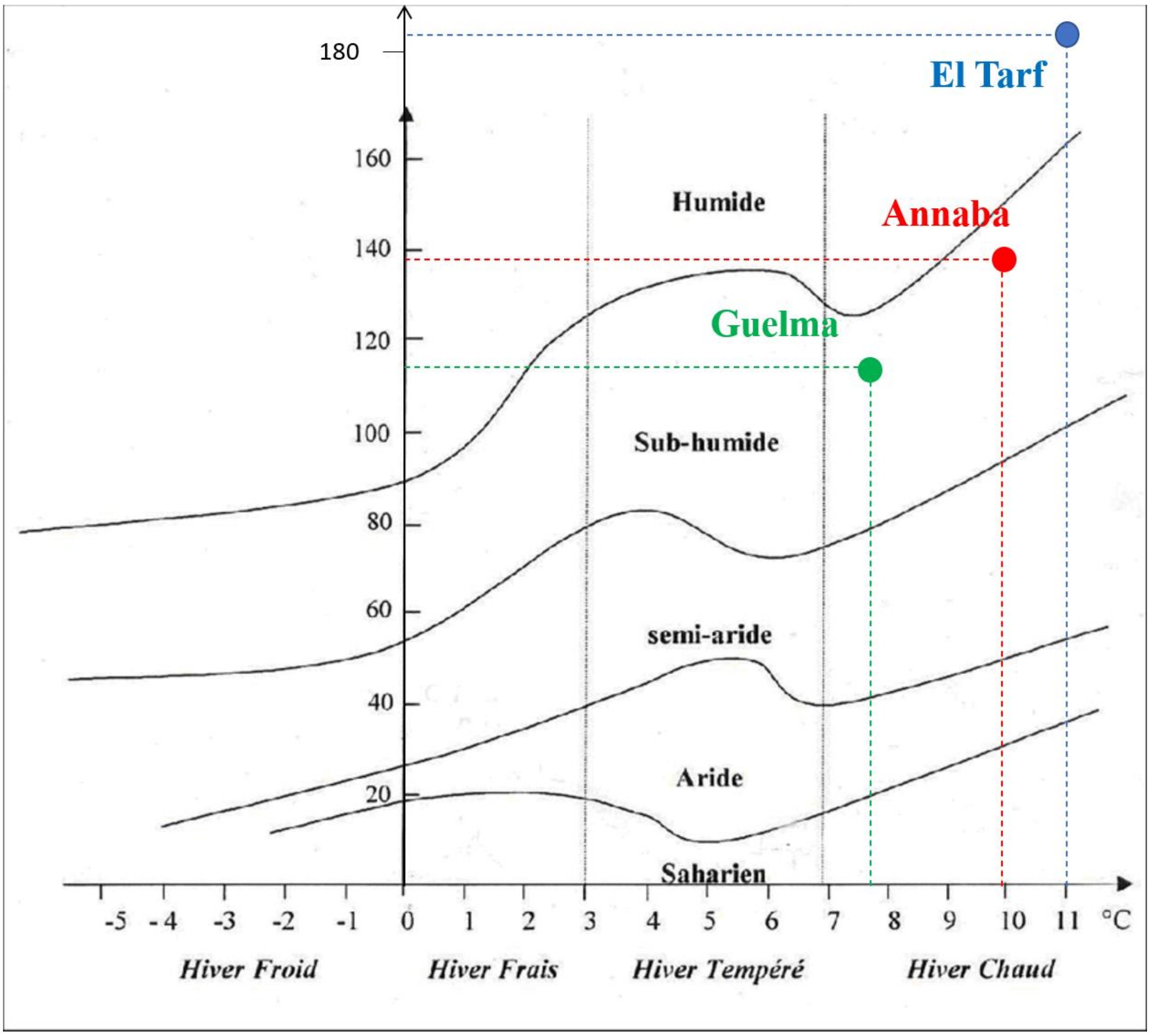


**Figure 04:** Location of Annaba, El Taref and Guelma regions on the Emberger climagram (1955)

### 1.3.5. Presentation and location of the study stations

Mosquitoes have an aquatic larval stage, so the diversity of aquatic sites between natural and artificial has been taken into consideration, and the choice of sites was made according to the impact of the climatic and geographical diversity of the sites studied.

A preliminary survey was carried out in natural and artificial breeding sites in the different regions of the wilayas of Annaba and El Tarf, which allowed us to inventory about larval sites where Culicidienne fauna was collected. We started our work during the period from March to October 2021. Where we collected samples from 20 larval habitats. This choice is depending on the function of the location, the presence of potential larval sites and the diversity of the environments.


**Figure 05**. types of the sampling sites (1: temporary pond - 2: permanent pond - 3: ditch - 4: tire)

## 2. Description of biological model:

Mosquitoes (Culicidae) are a family of Dipteran insects of the suborder Nematocera. They form a large group (more than 3500 species) with a practically worldwide distribution, since they are found in all temperate and tropical regions, even beyond the Arctic Circle. They would have made their appearance at the beginning of the Tertiary, since they have been discovered in Eocene and Oligocene rocks **(Trari, 2017).**

The Culicidae are divided into three subfamilies: Taxorhynchitinae, Anophelinae, Culicinae (**Figure 06**). The Culicidae family comprises about 3000 species **(Knight & Stone, 1977)**. In Algeria, 50 species of Culicidae from 6 different genera are grouped in the subfamilies Anophelinae and Culicinae **(Hassaine, 2002)**.

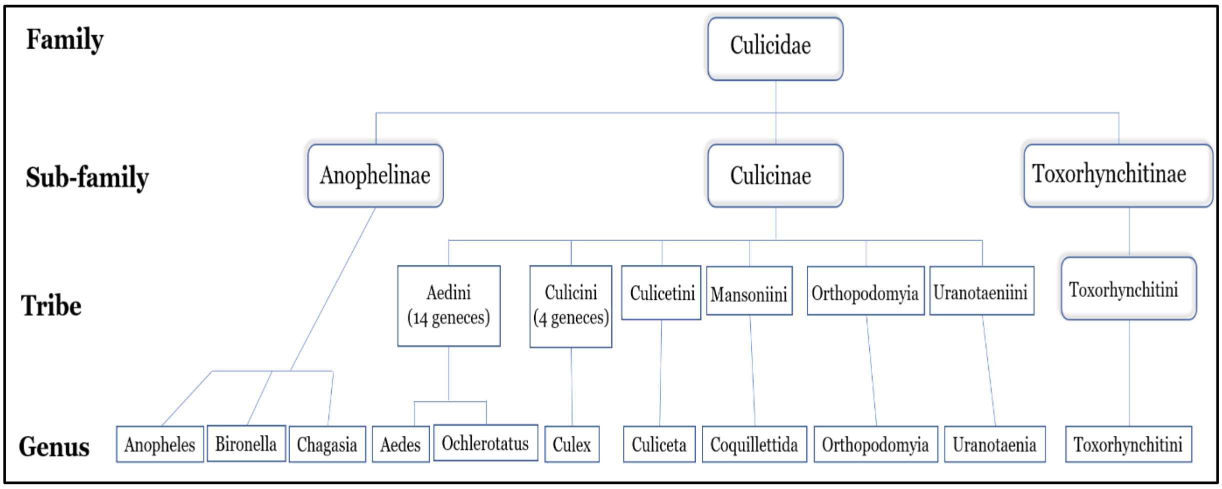


**Figure 06**: Simplified taxonomy of Culicidae **(Harbach, 2004)**.

### 2.1. Morphology and biology of Culicidae

Mosquitoes are small insects with long, thin wings; they differ from other Diptera, such as flies, in having small scales on most wing veins. They are holometabolous insects whose egg is ovoid, with a granular exochorion **(Lariviere & Abonnenc, 1956)**.

#### 2.1.1. Eggs

Female mosquitoes lay their eggs in stagnant water of ponds and ditches. Female *Anopheles* lays about 40-100 eggs at a time which are separate (not attached) and lie horizontally on the water surface (**Figure 07**). The eggs are spindle shaped having lateral air floats. *Culex* lays about 200-300 eggs at a time which are cigar shaped and without air spaces. The eggs are glued together from side to form ‘rafts’. The rafts are vertical with heavier end downward. The whole raft is concave and floats on the surface of water. The eggs stage lasts for about 2-3 days **(Humagain, 2018).**

**Figure 07**: A cluster of *Culex* eggs under a microscope **(1).**

**2.1.2. Larva:**

Worm like shaped with a body composed of head, thorax and abdomen. The head is broad and has two short bushy antennae on each side. It has two compound eyes, and mouth is situated ventrally on the head. A pair of long hair-like bristles called feeding brushes are present around the mouth which move rapidly to set up a current of water, carrying small food particles into the mouth. The thorax is slightly broader than the head. It has three pairs of lateral tufts of hairs, which protect from being eaten by fish (**Figure 08**).

The abdomen is 9 segmented; each segment bears a pair of lateral tufts. On the eighth segment, is located a long respiratory tube or siphon, which is thrusted above the water surface so that the larva can breathe. They also consist of 4 small leaf-like tracheal gills in the last abdominal segments which help them take $O_2$ dissolved in water. The larva swims actively in water by wriggling movement and hence is also called a wriggler. It is very active and feeds voraciously on microscopic algae and other creatures as it has mandibles and maxillae for biting and chewing. The larva stage lasts for about 2 weeks during which it molts 3-4 times and then develops into a pupa **(Humagain, 2018).**

**Figure 08** : Morphologie de la larve de *Culex pipiens.* **(2)**

**2.1.3. Nymphe:**

Pupa has a comma shaped body and can be divided into two parts: the swollen anterior part called cephalothorax and the posterior elongated hook like abdomen. The pupa is enclosed in a transparent membrane called puparium. The pupa of a mosquito is very active in comparison to the pupae of other insects. It swims vigorously by flicking its abdomen. It doesn't feed as it doesn't possess the mouth parts. They respire through a pair of small trumpet-shaped tubes called respiratory trumpets. The pupa stage lasts for 2-7 days during which the larval organs are broken down and the adult organs are formed **(Humagain, 2018).**

**2.1.4. Adult:**

The adult, once metamorphosed, causes a break in the nymphal head and emerges at the water surface. Males reach sexual maturity in one day while females which are larger than males, reach it in 1-2 days **(Clements, 1999)**.

The adult head has a pair of compound eyes, mouthparts and a pair of antennae. In males, these antennae are adorned with multiple long bristles that give a feathery appearance to these appendages. In females, they are shorter and less numerous (**figure 09) (Legros, 2014).**

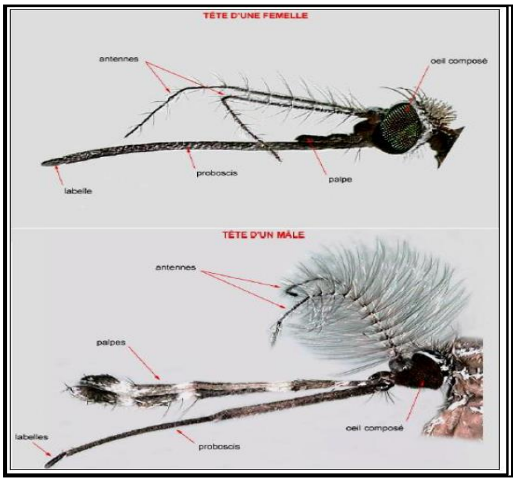


**Figure 09:** Sexual dimorphism of the male and female mosquito head **(Vacus., 2012)**.

The mouthparts are of the biting-sucking type **(figure 10)**. The proboscis is an odd organ located in the lower-medial part. Its structure differs between the sexes: in females, which are haematophagous, the proboscis is composed of three odd parts: the epipharynx, the hypopharynx and the labium, and four even and symmetrical parts, two mandibles at the top and two maxillae at the bottom. In males, which do not feed on blood but on plant juices, only the epipharynx and labium remain **(Legros., 2014)**.

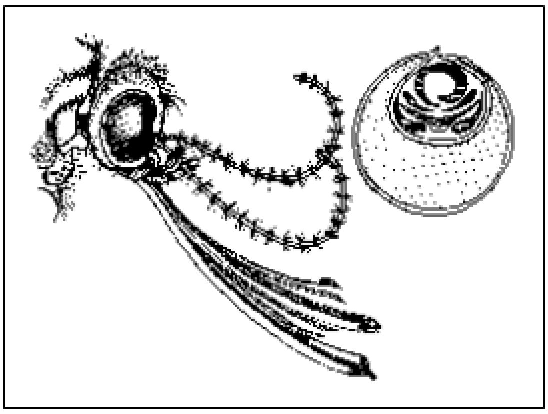

**Figure 10**: Mouth parts of a female mosquito. A: Labium, B: Labrum, C: Maxilla, D: Mandible, E: Hypopharynx, F: Maxillary palp, G: Labella, H: Antenna. **(3)**

Two features distinguish the male from the female with the naked eye; the palps are very short and tapered in the female, while in the male they are longer than the proboscis and her antennae are more developed and very hairy **(Urquhart *et al*., 1996; Euzeby, 2008)**.

It is 3 to 6 mm long.

- Palps elongated in the male (longer than the proboscis) and curved upwards.

- palpi shorter than the proboscis in the female (about a quarter of its size).

- at rest, the abdomen of adults is almost parallel to the support. (**Figure 11**)

**a. Head:**

The head is dark, covered with dark, erect forked scales with white scales and brown hairs between them. On the cheeks are shorter scales forming a white patch **(Kettle, 1995; Andreo, 2003).**

**b. Thorax:**

The thorax is brown with dark tan scales. The slender legs are brown and not ringed, the femur is black above and white below, and a white spot can be seen on the knee. The legs are made up of 5 pieces in total, and the tarsus, with 5 articles, has 2 claws. The wings are unmarked. They are also covered with scales attached to the veins and the posterior margin. At rest, they are folded over the abdomen. Behind the wings are the pendulums used for flight control **(Bussieras & Chermettes, 1991; Cachereul, 1997)**

**c. Abdomen:**

Slender and elongated, it is composed of 9 segments ending in 2 crests, short appendages protecting the anus and the genital opening. It is covered with light, brown and white scales with long hairs on the dorsal side. A dark longitudinal line and a few dark spots on the ribs adorn the ventral side. In males, the abdomen ends in a genital armature that serves to hold the female during mating. In females, there is an oviscapte which is used during oviposition **(Cachereul, 1997)**.

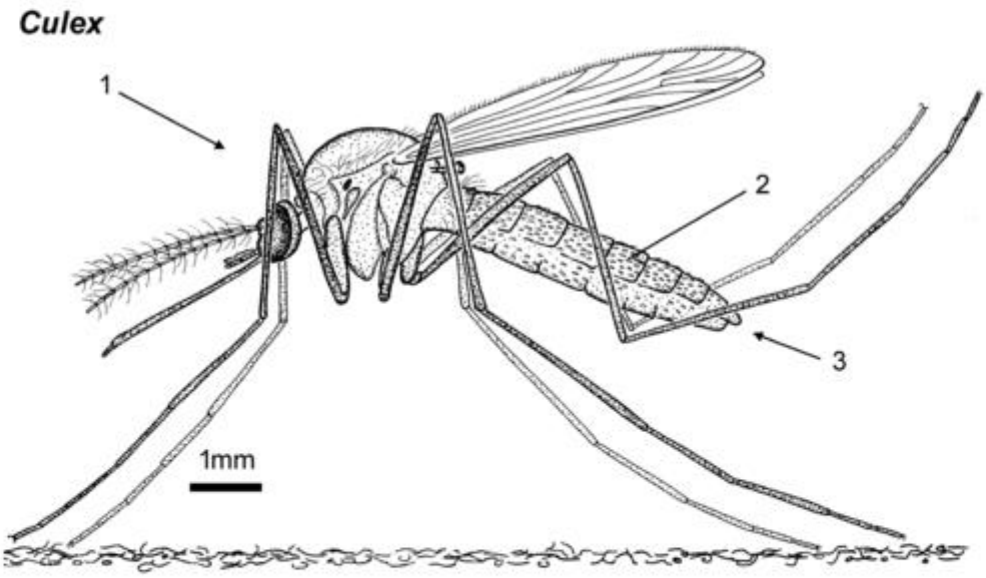


**Figure 11:** General morphology of an adult female of Culex species **(4)**

**2.2. Ecology and life cycle of mosquitoes:**

**2.2.1. Life cycle of mosquitoes:**

The development cycle of mosquitoes lasts approximately twelve (12) to twenty (20) days **(Adisso & Alia, 2005)**. Like all insects with complete metamorphosis (holometabolous), the development of the mosquito is characterised by two distinct phases **(Rodhain & Perez, 1985)**

a) the aquatic phase, which includes the first three stages.

b) the aerial phase which concerns the winged adult or imago **(Figure 12)**

**a) Aquatic phase:**

A few days after fertilisation, the eggs are laid by the female in different environments. Egg laying is often in the order of 100 to 400 eggs and the ovular stage lasts two (2) to three (3) days when the conditions: temperature of the environment, pH of the water, nature and abundance of aquatic vegetation as well as the associated fauna **(Kpondjo, 2008)** are favourable to hatching; this may be delayed, in the event of a drop in temperature for example **(Rodhain & Perez, 1985)**.

When mature, the eggs hatch into stage 1 larvae (1-2 mm) which, up to stage 4 (1.5 cm) feed on organic matter, micro-organisms and even live prey (for carnivorous species). The body is divided into three segments: head, stubby thorax without locomotor appendages and a flexible abdomen. Despite their aquatic evolution, mosquito larvae breathe through the air using respiratory stigmas or a siphon **(Rodhain & Perez, 1985)**. The stage 4 larva is clearly visible to the naked eye.

The nymph is generally comma-shaped, mobile and does not feed during the entire nymphal stage (metamorphosis phase) which lasts one (1) to two (2) days. It occasionally comes to the surface of the water to breathe. At the end of this stage, the pupa stretches, its tegument splits dorsally and, very slowly, the adult mosquito (imago) emerges from the exuviae: this is emergence, which lasts about fifteen (15) minutes during which the insect is exposed without defence to numerous surface predators **(Rodhain & Perez, 1985)**.

**b) Aerial phase:**

Both sexes mate in flight or in vegetation and have a flight distance of one (1) to two (2) km, depending on the species. Thanks to the long hairs on their antennae, the males can perceive the buzzing sound produced by the rapid beating of the wings of the females, which approach the tests during the nuptial flight. At this time, the male fertilises the female by leaving a supply of his seed with her. The female has a particular trait, that of keeping the sperm alive until it dies, and she stores the male's semen in a globular ampulla or storage vesicle (sperm cell). It therefore mates only once **(Darriet, 1998)**.

The males feed on nectar for energy. Only the females are haematophagous. They do not need blood for their own survival, but take the proteins necessary for the maturation of their eggs. After the blood meal taken from a vertebrate (mammal, amphibian, bird), the female is then digested in a sheltered place **(Guillaumot, 2006)**. As soon as the female is pregnant, she starts looking for a suitable oviposition site for the development of her larvae. Oviposition usually takes place at dusk. The larval site is stagnant or slow-moving water, fresh or salt **(Ayitchedji, 1990)**.

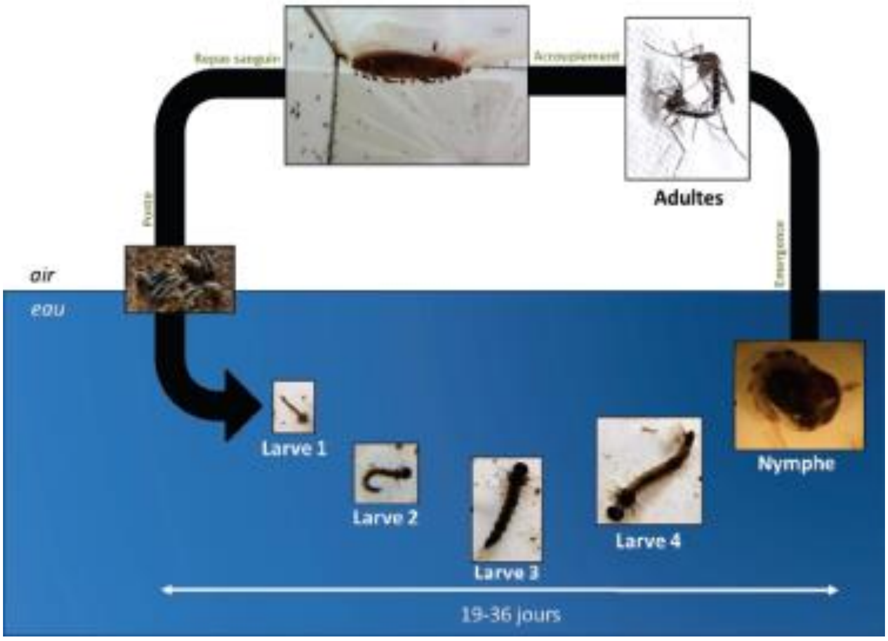


**Figure 12**: The mosquito development cycle. (**Raharimalala *et al*., 2012**)

#### 2.2.2. Interest of Culicidae in the ecosystem:

##### a) Mosquitoes that pollinate:

There are about 3,500 mosquito species, many of which want nothing to do with biting humans or any other animal. Even in species that bite, it is only the females that do so and just to develop their eggs.

The fundamental food of all adult mosquitoes is plant sugar and its associated nutrients, most often in the form of floral nectar. In the process of looking for nectar, mosquitoes pollinate many of the flowers they visit — this is one of the most commonly overlooked ecological functions of mosquitoes.

Mosquito pollination is likely far more common than we realize. There is evidence that mosquitoes function as generalist pollinators in some plant families, and there are many known instances of mosquito pollination that are simply overlooked.

Mosquito pollination was observed as far back as the 19th century. Mosquito pollination is hard to see, as most mosquitoes visit flowers near or after dusk and human presence disturbs mosquitoes from nearby flowers. In the Arctic, plants make use of vast hordes of nectar-hungry mosquitoes for pollination during the short growing season **(Peach, 2019)**.

**b) Biomass transfers:**

Mosquito larvae grow by consuming microorganisms such as algae and microbes that decompose decaying plant material. Larval mosquitoes contribute to aquatic food chains by serving as food sources for many predators, including fish, amphibian and larvae of dragonfly **(Peach, 2019)**.

**c) Disease reduction, ecosystem balance:**

Mosquitoes are also the world's deadliest animal and cause immense suffering. Ideally, we should maintain the ecosystem functions of mosquitoes while also reducing disease burden.

Not all mosquito species are responsible for spreading pathogens. Targeting specific species or making the mosquitoes themselves immune to pathogens and thus unable to spread them would protect humans while keeping the ecosystem function of mosquitoes intact.

In a world of collapsing ecosystems and declining pollinator populations we need all of the help we can get. This includes acknowledging the secret lives of mosquitoes and more sophisticated mosquito control strategies that protects their ecosystem functions **(Peach, 2019)**.

**2.3. Medical and veterinary interest of Culicidae:**

**2.3.1. Female bite:**

In both humans and animals, the bite of the female mosquito causes a round lesion a few mm to 2 cm in diameter, often pruritic.

Allergic reactions to these bites may occur, due to the injection of salivary antigens, but may also be due to simple contact with the mosquito or its excrement **(Candace *et al*., 2001)**. The expression of this allergy in dogs can be both local and generalised, and manifests as highly pruritic erythematous patches **(Prelaud, 1991)**.

**2.3.2. Transmission of diseases:**

Mosquitoes are vectors of many diseases **(Chauve, 1990; Andreo *et al*., 2003)**. In general, the transmission of pathogens follows a cycle that varies little: contamination of the mosquito on a host 1 carrying the disease, maturation and sometimes multiplication of the pathogen in the body of the mosquito, and then inoculation to a host 2 during a second blood meal. The main pathogens are:

**a) viruses:** West Nile, yellow fever, dengue…

**b) Parasites:** including wire-borne diseases:

- *Wucheriabancrofti* : responsible for lymphatic filariasis in humans.
- *Dirofilariaimmitis:* responsible for cardiopulmonary heartworm disease in dogs, but also in coyotes, wolves, foxes, cats, ursids and pinnipeds **(Euzeby *et al*., 1990).**

Embolisation of parasites in the pulmonary artery can lead to endarteritis and fibrosis of the arterial intima **(Chauve, 1990; Davoust., 1994)**.

Decompensated heart disease may be observed in some severe forms. The larvae, or microfilariae, pass into the bloodstream, from where they can infect mosquitoes taking their blood meal. After maturing for 15 days inside the mosquito, they can become infesting and infect a new animal during a second blood meal **(Giraud, 1994)**.

➢ **Paludisme**

Dengue is by far the most important arbovirosis and a major public health problem in most tropical and some subtropical regions. According to the most recent estimates, about 390 million people are infected each year and 96 million show symptoms of the disease **(Bhatt *et al*., 2013)**. (Figure 13)

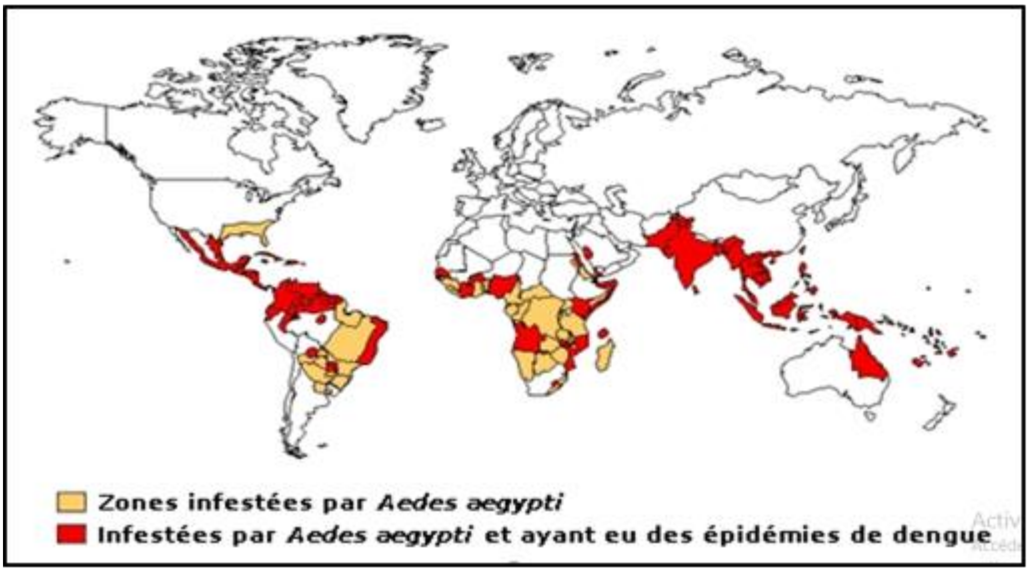


**Figure 13:** Distribution of dengue worldwide **(5)**

➢ **Chikungunya :**

Chikungunya, from Makonde (a Tanzanian dialect) "the man who walks hunched over", is an arboviruses transmitted to humans by mosquitoes of the genus Aedes (Ae. spp. including *Ae.Aegypti* and *Ae. albopictus*). The name reflects the characteristic posture of chikungunya patients, due to the joint pain it causes **(Duvant & Robillard, 2007)**.

➢ **Zika :**

Zika virus (ZIKAV) is a flavivirus transmitted primarily to humans by Aedes mosquitoes. Other documented modes of transmission include sexual, perinatal, laboratory exposure and probably blood transfusion **(Melo *et al*., 2016, Russell *et al*., 2017)**. Most ZIKAV infections are asymptomatic. **(Duffy *et al*., 2009)**

It is usually mild and accompanied by clinical symptoms: maculo-papular rash, fever, arthralgia and/or non-purulent conjunctivitis. However, ZIKAV infection during pregnancy can lead to adverse effects, such as fetal loss, congenital microcephaly or other severe brain abnormalities **(Melo *et al*., 2016).**

ZIKAV was first isolated in 1947 in the Zika forest in Uganda from a sentinel rhesus monkey in a yellow fever research study **(Dick *et al*., 1952)**. **(Figure 14)**

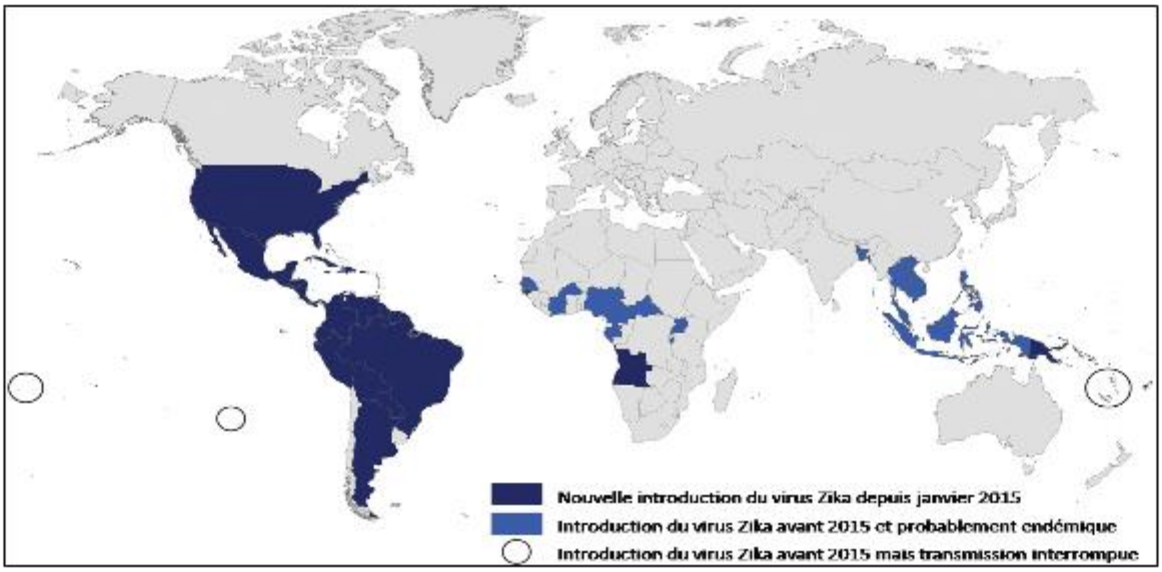


**Figure 14:** Countries and territories with evidence of current or past Zika virus transmission **(Hills *et al*., 2017)**

### 2.4. Vector control methods:

Based on mosquito control, they consist of mechanical (physical), chemical, biological and genetic controls.

- **Mechanical control :**

It aims to limit the proliferation of insect vectors and reduce human-mosquito contact. It is done by eliminating potential mosquito breeding grounds around human dwellings (draining and filling in marinas, digging depressions, etc.), using impregnated mosquito nets, and maintaining **habitats (Carnevale & Mouchet, 1999 in Kone, 2009).**

- **Physical control**

It is an intentional modification of the biotope, which aims to remove or reduce by physical means the surface water bodies in which mosquitoes thrive. Physical action is usually undertaken to regulate the water regime, improve flow, or otherwise physically alter the water body **(WHO, 1999)**.

- **Chemical control**

Most mosquito control measures are based on chemical control using insecticides. Depending on the case, either antilarval measures (dispersal of insecticides in breeding sites) or adulticidal techniques (house spraying) may be adopted. Chemical control is achieved by using synthetic or plant-based products that kill the insects by ingestion or contact. The method of application of products depends on the ecology of the vector and the stage targeted **(Nosais, 1996 in Kone, 2009)**. Insecticides used against mosquitoes include different molecules belonging to several chemical families (Organophosphates, carbamates, perythrinoids, bioinsecticides, etc.) with different modes of action **(Goislard. 2012)**.

- **Biological control**

This involves the introduction of different species of organisms that are enemies of mosquitoes into their biotope. These include the larvivorous fish (Gambusia affinis), whose action is limited to permanent waters, and the bacterium Bacillus **(Margot. 2010)**, which causes mortality in mosquito larvae of the genera Culex and Anopheles to a lesser degree on Aedes. Herbivorous fish (carp) are used in China to devour grasses that provide shelter for mosquito larvae **(Wu *et al*., 1991 in Kone, 2009)**.

- **Genetical control**

It involves the manipulation of the genetic make-up of mosquitoes to produce transgenic individuals that may be either sterile or resistant to the parasites they normally transmit. Manipulations also involve plants such as algae that reproduce in breeding grounds. These algae, genetically modified by integrating bacterial toxin genes, act on mosquito larvae **(Tabachnick, 2003 in Kone., 2009)**.

**3. Working methods:**

**3.1. Harvesting method**

The study was carried out between April 2021 and March 2022 in three Wilaya of the country (Annaba, El Tarf and Guelma). The methods used for inventory were different depending on the data necessary: We used two methods one is a prospering of the Culicidae fauna in north east of Algeria collecting larvae from both natural and artificial sites using the dipping method **(Awono-Ambéné & Robert, 1999)**.

The second study was using the ovitrap method developed in 1965. Oviposition traps were used for Aedes populations that shown to be effective for larval research and studies on vector frequency **(Fay & Eliason, 1966; Regis *et al*., 2008).** Our ovitraps were placed in specific sites and visited every fortnight (a total of 24 samplings per year) to collect larvae, in order to provide an attractive spawning site. These traps were stable in time and space and contained water at all times, located in an environment that was itself attractive to mosquitoes: El Bouni (36°51,010'N; 007°43,900'E) in the region of Annaba; Besbes (36°29,902'N; 007°43,060'E) in the region of El Tarf; and Bouchegouf (36°42,140'N; 007°50,681'E) in the region of Guelma. appears in the card.

The trap consists of a black plastic bucket with a volume of 10 litres according to **Marabuto & Rebelo, (2018) (Figure 15)**. These were placed in hut gardens, as the preferential roosts of *Aedes albopictus* are not exposed to sun and wind, **Hawley (1988)**. In addition to monitoring the *Aedes albopictus* population, this method has been a tool to detect other mosquito species capable of cohabiting with it, resulting in a comparative study.

**Figure 15.** Model of the ovitrap used **(Rouibi *et al* 2023)**

**3.2. Identification of collected species:**

Mosquito larvae were collected from potential breeding sites, brought back to the laboratory, some killed, preserved in alcohol and identified, and some reared to adult stages. The latter were captured twice a month for each study station, depending on the weather conditions and the season.

To confirm that our breeding of each of the two target species is sound and accurate, they are identified using identification software. Only larvae that have reached the fourth instar are reliably identified. The larvae are placed in glycerine on a slide to be observed under a microscope with different objectives. The identification of larvae and adults is referred to dichotomous keys mainly those of **Rioux (1958)**; **Himmi *et al* (1995))** and the use of two identification software programs, made by **(Brunhes *et al*. 1999)** for Mediterranean African mosquitoes and **(Schaffner *et al*. 2001)** for European mosquitoes.

The slide preparation and mounting technique, which is similar to that used by **(Matile, 1993)** for fourth instar larvae only, provides reliable identification. The larvae are preserved in 70° glycerol ethyl alcohol and grouped by station at the time of mounting. For adult identification, after collection, the mosquitoes caught in the net are transferred to tubes to facilitate sorting of males and females, using very fine pins or rigid paper triangles, which are fixed for better handling during identification.

### 3.3. Breeding of mosquitoes in the laboratory:

The samples found in the inventory helped us collect both *Aedes albopictus* and *Culex pipiens* from the region of Boussedra and El Bouni (Annaba-Algeria). Adult mosquitoes were reared in cubic cages (32x32x32 cm) covered with a mosquito net in a room where the temperature was maintained at 25°C ± 2, a hygrometry of 80% ± 5 and a photoperiod of 12 hours during the day and 12 hours at night **(Figure 16)**.

Adults are fed by dates attached to the top of the cage in a cluster. Breeding individuals are additionally fed a blood meal by exposing pigeons to females in a cage for 15 minutes twice a week **(Trari *et al*, 2002)**. Female *Ae. albopictus* mosquitoes lay eggs on the edges of boxes **(Figure 17)** containing 250 ml of spring water and on wet stones. However, female *Cx.pipiens* mosquitoes lay their eggs in clusters. After the eggs have been laid, the box is replaced by a new one and the box containing the eggs is placed in another cage for the rearing of the larvae for toxicological treatments.

After 24 to 72 h, the first larvae hatch and are fed with 0.04 g of the 75% biscuit - 25% yeast mixture **(Rehimi & Soltani, 1999)**. The water in the containers containing the larvae is renewed every three days. After moulting, the pupae are transferred to a cage where they can undergo metamorphosis to the adult stage.

**Figure 16.** Breeding cages at the eco-ethology laboratory level. **(Original)**

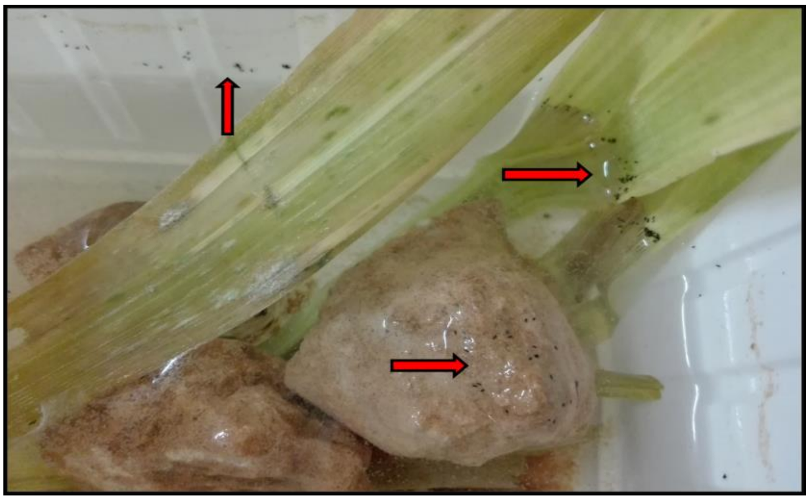

**Figure 17.** *Aedes albopictus* eggs on the edges of boxes and on wet stones. **(Original)**

### 3.4. Preparation of bioinsecticides for larval treatment

We used in toxicological study an insecticide called Spinosad, which is a naturally occurring insecticide, is a member of the spinosoid family. Indeed, it shows low toxicity to humans, mammals, aquatic invertebrates and non-target organisms (Kirst, 2010) and is environmentally friendly due to its rapid biodegradation and high selectivity. Spinosad has a novel and unique neurotoxic mode of action as it acts on both nAChRs **(Kirst, 2010; Rinkevich & Scott, 2012)** and GABAergic receptors **(Ishaaya., 2001)**; it acts by contact or ingestion, and is highly effective against Lepidoptera and Diptera.

Spinosad is of microbial origin and is derived from the aerobic fermentation of an Actinomycete bacteria Saccharopolyspora spinosa. After fermentation, Spinosad is extracted and formulated into a concentrated white crystalline aqueous suspension **(Hansen, 2002)**.

### 3.5. Preparation of larvae for a control test:

For the purpose of a bio-pesticide control trial, four 100 ml containers are prepared (80 ml of rainwater or spring water + 20 ml of the studied insecticide), and in each container 20 L4 stage larvae of *Culex pipiens* are introduced. In the four containers used, treated batches with three replicates for each concentration used and control batches are noted to observe the differences between the bio-insecticide treated larvae and the control larvae, and also to make a real time comparison of the mortality within all batches.

After preliminary tests, we determined three doses to be administered to L4 larvae of *Culex pipiens* by Spinosad (0.125g/l, 0.25g /l, 0.5g/l).

**4. Statistical analysis:**

**4.1. Methods of exploitation and synthesis of results:**

The factors that determine the distribution of species are of two groups:

- Intrinsic (a species' ability to spread, its ecological range, its reproductive potential)

- Extrinsic (geography, climate, edaphic and biotic conditions).

The distribution of Culicidian species is thus the result of the evolution of these factors **(Lacoste & Salanon, 1969)**.

Communities are associations of populations, their characteristics are strongly influenced by the physical environment and the interactions between populations. They differ in their number of species (species richness) and in the relative abundance of these species, a characteristic referred to as species diversity **(Robert *et al*.2005)**.

In a pre-Saharan territory such as Biskra, the species are not uniformly distributed in all the surveyed stations (larval sites or plant composition). For this reason we proposed to exploit our results by two methods of ecological indices presented also by **Daget (1976)** and **Southwood (1978)**.

The ecological indices studied in our study are: the composition index (total and average richness, frequency, constancy), the Shannon-Waever index and the equispersion index.

**4.1.1. Ecological composition indices:**

The ecological composition indices used are specific and total richness, mean richness, centesimal frequency (CF) or relative abundance (RA) and occurrence index.

**a) Total species richness (S)**

By definition, total richness is the number of species in a given stand in a given ecosystem **(Ramade, 1984)**. It represents one of the fundamental parameter's characteristics of a stand **(Muller, 1985)**. According to **Benyacoub and Chabi (2000)**, richness is the total number of species found during a series of (n) surveys in an environment. For the present study, total richness is the total number of species obtained from the total number of surveys.

The total richness of a stand **(Blondel, 1975)** is the number of species (S) found in the study area, while the average richness (S') is the quotient of the total number of individuals (Ki) for each species over the total number of surveys (N) carried out, i.e.:

$$S' = K_i / N$$

**b) Richness mean (Sm):**

The average richness corresponds to the average number of species present in the samples of a studied stand. The average richness (Sm) is very useful in the study of the structure of stands, it corresponds to the average number of species contacted in each survey **(Ramade, 1984)**. According to **Blondel (1979)**, the average richness is equal to :

ni : number of species in survey i.

R : total number of surveys.

$$\mathbf{Sm} = \frac{\sum_{i=1}^{R} n_i}{R}$$

**c) Relative abundance:**

The abundance of an organism is the total number of that organism or the number of organisms per unit space. The second definition refers to the population density of the organism. Abundance, together with distribution, is a basic measure in ecology. Both concepts reflect the influence that biological and environmental factors have on an organism **(Claude & Christiane, 2003; Faurie *et al*., 2008)**.

Relative abundance is the percentage of individuals of the species (ni) in relation to the total number of individuals (N) of all species combined **(Dajoz, 2000)**. It is calculated as follows:

$n_i$ : number of individuals of a species i.

N : total number of individuals of all species

$$A.R\ (\%) = \frac{n_i \times 100}{N}$$

**d) Constancy or index of occurrence:**

Consistency is expressed as the number of records containing the species of interest in relation to the total number of records **(Dajoz, 1982)**. Consistency is calculated by the following formula:

$p_i$ : number of surveys containing the species studied.

p : total number of surveys conducted.

$$C\ (\%) = \frac{p_i \times 100}{p}$$

Depending on the value of C, the following categories are distinguished:

**Table 06.** Categories of constancy According to **Dajoz (1971)**.

| Constancy (%) | Categories |
|---|---|
| 100 | Omnipresent |
| 75 % ≤ C < 100 | Constant |
| 50 % ≤ C < 75 | Frequent |
| 25 % ≤ C < 50 | Accessory |
| 5 ≤ C < 25 | Accidental |
| ≤ 4 | Rare |

**4.1.2. Ecological structure index**

The structure indices show the qualitative aspect of the studied entomofauna. They are the Shannon-Weaver diversity, equipartition, concentration and uniformity index and the abundance distribution applied to the **Motomura (1932)** log-linear model. The various diversity indices currently in use make it possible to study the structure of populations with or without reference to a concrete spatio-temporal framework. They provide a rapid assessment of stand biodiversity in a single figure **(Jacques & Christian, 2003)**.

**a) Specific diversity:**

Diversity can be expressed in terms of the number of species present in an environment, but this number is not always known exactly. In this case, various diversity indices are proposed to compare populations between them and to see how they evolve in space and time. In the interpretation of our results, we used the Shannon and Weaver (1963) diversity index. This index is defined as the probability of occurrence of an event and calculated according to the following formula **(Ramade, 1984)**:

$$H' = - \Sigma P_i \log_2 P_i \qquad \text{Où} \qquad P_i = n_i / N$$

ni : number of individuals of a species i.

N : numbers or total number of individuals in the collection.

The value given by this formula is information expressed in bits. Diversity varies not only according to the number of species presented but also according to their relative abundance **(Barbault, 1981)**. It is highest when all species in the stand are represented by the same number of individuals. On the other hand, if diversity is low, we speak of a stand with low species diversity **(Blondel, 1979)**.

**b) Equitability:**

The equitability index (E) is the ratio of observed diversity (H') to maximum diversity (H' max) **(Weesi and Belemsobgo, 1977)**. It is calculated using the following formula.

$$H'_{max} = \log_2 S$$

S : total richness

$$E = \frac{H'}{H'_{max}}$$

This index varies between 0 and 1. It tends towards 0 when almost all of the population corresponds to a single species in the stand. It tends towards 1 when each species is represented by the same number of individuals **(Ramade, 1984)**.

**c) Concentration and uniformity:**

**Simpson (1949)** proposed a concentration index (C), which gives the probability that a second individual drawn from a population would be of the same species as the first. We will use this formula in our results.

n: number of species.

n i: number of individuals.

$$\mathbf{C}= \frac{\sum_{i=1}^{n} n\,(ni-1)}{N(N-1)}$$

N: number of individuals of a species i.

Following the previous author, **Greenberg (1956)** proposes another formula to measure specific diversity (D): **D = 1 – C**

C : Concentration.

According to **Daget (1976)**, with diversity indices it is possible to compare the structure of several stands and their variation only in space.

**d) Abundance distribution:**

If diversity gives us a population structure by means of numbers, it is therefore necessary to have precise knowledge of the population structure by studying the specific abundance distribution of species.

For this purpose, this distribution was studied by using appropriate mathematical models to represent this distribution.

Our study is conducted using the **Motomura (1932)** or log-linear model, which allows us to understand the spatial distribution of species and the structure of mosquito populations, the graphs represented and the calculations of the lines are carried out using the Exel-stat-2015 software.

**4.2. Statistical method of exploitation of results:**

Data processing for distribution of abundance, analysis of variance and factorial correspondence analysis is carried out using Exel-Stat 2015.

**4.2.1. Analysis of variance**

The comparison tests between the three Culicidae larvae sites are carried out by one-factor analysis of variance.

Analysis of variance (A.N.O.V.A) is the arithmetic mean of the squares of the deviations from the mean. Its purpose is to compare the averages of several populations assumed to be normal and of the same variance, based on simple random samples that are independent of each other **(Dagnelie, 1975)**.

ANOVA is used to test the homogeneity of a set of samples. ANOVA is used to show whether there is a significant difference. The P-value is compared with $\alpha = 0.05$. If $p \leq 0.05$, then there are significant differences; if $p > 0.05$, then there is no significant difference **(Berk & Steagall, 1995)**.

**4.3. Analysis of toxicity results :**

With regard to the results obtained for the toxicological study, the lethal concentrations (LC50% and LC90%) for the different pesticides used were calculated according to the mathematical procedures of **Finney (1971)**.

During the three days of exposure to the insecticide, the variable measured is the number of dead individuals per day. The observed mortality rate is corrected by Abott's formula, which gives the actual toxicity of the insecticides. The different rates are angularly transformed according to the Bliss tables. The data are thus standardized and subjected to an analysis of variance on XLStat 2015, then transformed into probits, which makes it possible to establish a regression line according to the decimal logarithms of the concentrations used, and the $\chi^2$ test allows a good fit of the line **(Finney, 1971)**. From this line the lethal concentrations were calculated.

#### 4.3.1. Observed mortality:

The percentage of observed mortality (O.M.) of control and treated larvae of the test individuals was determined according to the following formula:

$$\text{O.M} = \frac{Number\ of\ dead\ individuals\ after\ treatment}{Ntotal\ number\ of\ individuals} \times 100$$

#### 4.3.2. Corrected mortality:

The percentage of mortality observed in the treated larvae is transformed to corrected mortality (C.M.) according to the formula of **Abbott, (1925)**, which eliminates the natural mortality, which must be between 4 and 16%, and which is recorded in the control series.

$$\text{M.C} = \frac{\text{Mortality observed in treated lots} - \text{Mortality observed in control lots}}{100 - \text{Mortality observed in control lots}} \times 100$$

#### 4.3.3. Angular transformation:

The percentages of corrected (or observed) mortality undergo an angular transformation according to the method of **Fisher & Yates (1938).** The data obtained were analysed by ANOVA (Analysis of Variance with one classification criterion) to determine the significance level (*P*) using the software, XlStat 2015.

**4.3.4. Probit analysis:**

The regression line of decimal log concentrations (X) versus probits (Y), derived from the angular transformation of the corrected mortality means according to **(Fisher & Yates 1938)**, allows the estimation of the two lethal doses LD50 and LD90 according to **(Finney 1971)**, as follows:

$Y = a X + b$ so $X = \frac{Probit\ X - b}{a}$ or Y = probit 50 (90) and X=log LD 50 (LD90)

Anti log X= LD50. Also only for LD90.

The confidence interval (lower and upper limit) of these two lethal concentrations (LC50 and LC90) was calculated according to the method of **(Swaroop *et al*., 1966)** as follows:

Higher limit = CL50 × FCL50

inferior limit = CL50 / FCL50

FLC50 = Anti log C or $C = 2.77\sqrt{N \log S}$ and $S = \frac{LC84/LC50 + LC50/LC16}{2}$

N: Number of dead nymphs between LD16 and the LD84; S: Slope.

# II. Results

## 1. Culicidae inventory results

### 1.1. Quantitative importance of the Culicidian fauna in the Northeast :

A total of 2248 individuals were collected for the whole study period, of all the 21 stations found positive. We found five stations in the Wilaya of Annaba with 1407 specimen, in the Wilaya of Guelma we collected 272 individuals and finally El Tarf with 569 individuals.

### 1.2. Qualitative importance of Culicidae fauna by station:

All the species recorded in the study stations during the 2021 survey year: Annaba, El Tarf, and Guelma are shown in **Table. 07**. The examination of the results shows the existence of 9 species of Culicidae belonging to two subfamilies, that of Culicinae, where the presence of three tribes was noted: the tribe of Aedini is represented by three species: *Aedes caspius, Aedes geniculatus*, *Aedes albopictus*. The second tribe is the Culicini, which is represented by four species: *Culex pipiens*, *Culex hortensis*, *Culex theileri*, and *Culex perexiguus*. The tribe of Culisetini was presented by a single species: *Culiseta longiareolata*.

The second sub-family is the Anophelinae, which has been presented by a single species: *Anopheles labranchiae.*

The analysis of the species composition of Culicidae in the different study stations shows first of all that each environment has a particular fauna.

**Table 07**: Systematics of identified species

| Families | Sub families | Tribu | Genus | Sub genus | Species |
|---|---|---|---|---|---|
| Culicidae | Anophelinae | Anopheleni | Anopheles | Anopheles | *Anopheles labranchiae* Falleroni, 1926 |
| | Culicinae | Aedeni | Aedes | *Ochlerotatus* | *Aedes caspius* Pallas, 1771 |
| | | | | *Aedes* | *Aedes geniculatus* Olivier, 1791 |
| | | | | | *Aedes albopictus* Skuse, 1895 |
| | | Culicini | Culex | *Culex* | *Culex pipiens* Linné, 1758 |
| | | | | | *Culex hortensis* Linnaeus, 1758 |
| | | | | | *Culex theileri* Theobald, *1903* |
| | | | | | *Culex perexiguus* Theobald, 1903 |
| | | Culisetini | Culiseta | *Allotheobalda* | *Culiseta longiareolata* Aitken, 1954 |

**Table 08:** Distribution of Culicidae inventoried in the selected sites in the study areas.

| Species | Annaba | El Tarf | Guelma |
|---|---|---|---|
| ***Anopheles labranchiae*** | 1 | 1 | 1 |
| ***Aedes caspius*** | 0 | 0 | 1 |
| ***Aedes geniculatus*** | 0 | 0 | 1 |
| ***Aedes albopictus*** | 1 | 1 | 0 |
| ***Culex pipiens*** | 1 | 1 | 1 |
| ***Culex hortensis*** | 0 | 1 | 0 |
| ***Culex theileri*** | 0 | 1 | 1 |
| ***Culex perexiguus*** | 1 | 0 | 1 |
| ***Culiseta longiareolata*** | 1 | 1 | 1 |

The spatial distribution of Culicidae species in our study area and in the different types of nests is interesting for the study period. The results corresponding to this distribution are shown in **Table. 08** where the symbols (1) and (0) indicate respectively the presence and absence of the species in the three Wilaya of study.

It appears from this table that three species are cosmopolitan and are present in all the Wilaya: *Cx pipiens*, *Cs longiareolata* and *An labranchiae*.

Other Culicidae species were found in only a few stations, the case of : *Ae caspius Ae geniculatus Ae albopictus*, *Cx thieleri*, *Cx perexiguus*, and *Cx hortensis.*

A ranking of the centesimal frequency rates according to the number of identified species was done, which ranked *Culex pipiens*, *Aedes albopictus* and *Culiseta logiareolata*, as the three most identified species with a rate of 41.6%, 33.1% and 20.5% successively. *Anopheles labranchiae* with 1.9%, followed by *Culex perexiguus* with 1.02%. For the other species, the rates were very low and below 1% (**Figure 18, Figure 19**).

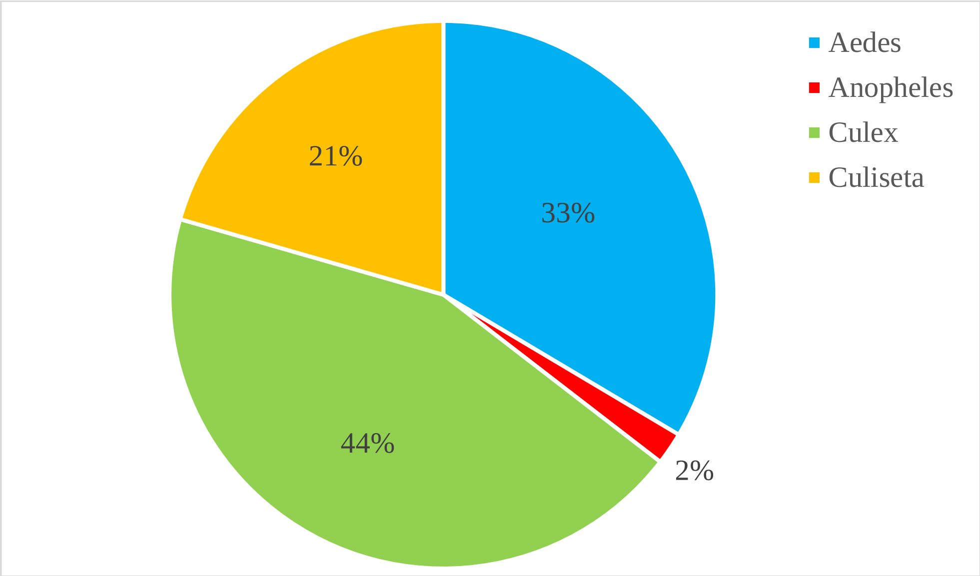


**Figure 18:** The centesimal frequency of Culicidae genera.

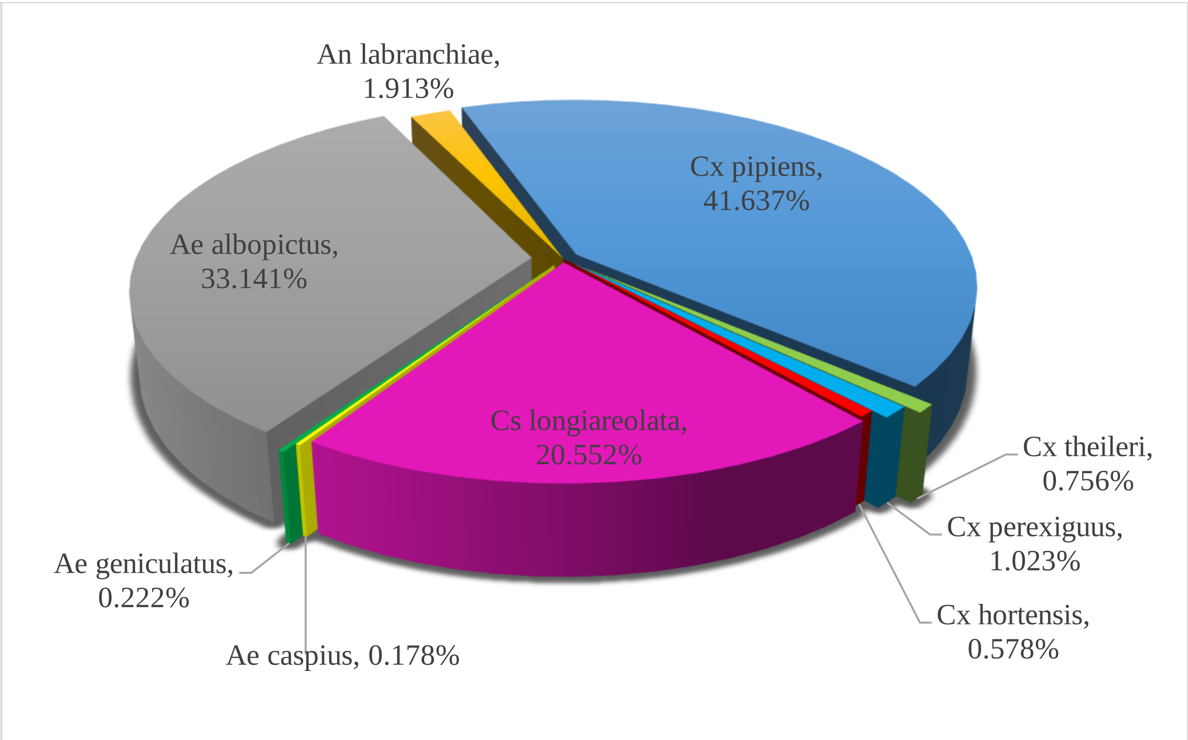


**Figure 19:** The centesimal frequency of Culicidae species.

### 1.3. Systematic features of some identified species:

#### 1.3.1. *Aedes albopictus:*

The adult specimen identified wears white stripes on its legs (**figure 20-B**), and what shows us that it's *Aedes albopictus* is the presence of a white longitudinal line on its black thorax, visible to the naked eye. Both sexes have the same general morphology except for the antennae and mouthparts.

The eggs, spindle-shaped, are black in colour and lack lateral floaters (**figure 20-A**). They are approximately 1mm in length with thick shell that allows significant resistance to desiccation. We found them separated such as every Aedes species.

The larvae of Aedes albopictus, measuring 2 to 12 mm, is worm-shaped, cylindro-conical and legless (**figure 20-C and D**). It's very similar to that of *Aedes aegypti* but it is light brown in colour.

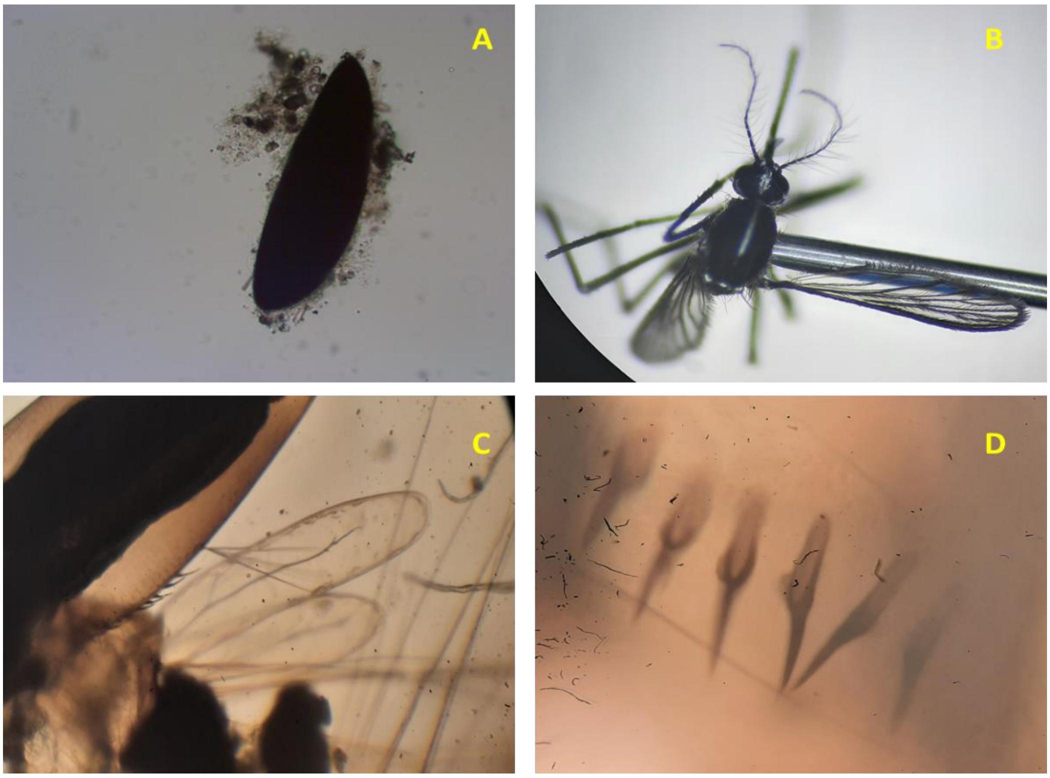


**Figure 20:** Morphological characteristics of identification of *Aedes albopictus.*

**A:** egg **B:** The female adult of Ae albopictus **C:** Siphonal tufts **D:** shape of comb teeth on segment VIII

### 1.3.2. *Culex pipiens:*

It is a multivoltine species, very abundant during the summer and autumn months. The female imagos overwinter in cellars, stables, caves and other natural and artificial shelters.

The eggs are deposited on the surface of the water and assembled in trays of 240 to 340 eggs, 30 to 40 for the autogenous ones. The larvae develop in water that is highly polluted by organic matter (waste water drainage pits, temporary pools, etc.). They can also be found in nests with fresh, pure water. The females bite all warm-blooded vertebrates at night, they take their meals mainly inside houses. (Schaffner et al, 2001). The species is a vector of West Nile virus, Sindbis and an avian plasmodium (**Figure 21**).

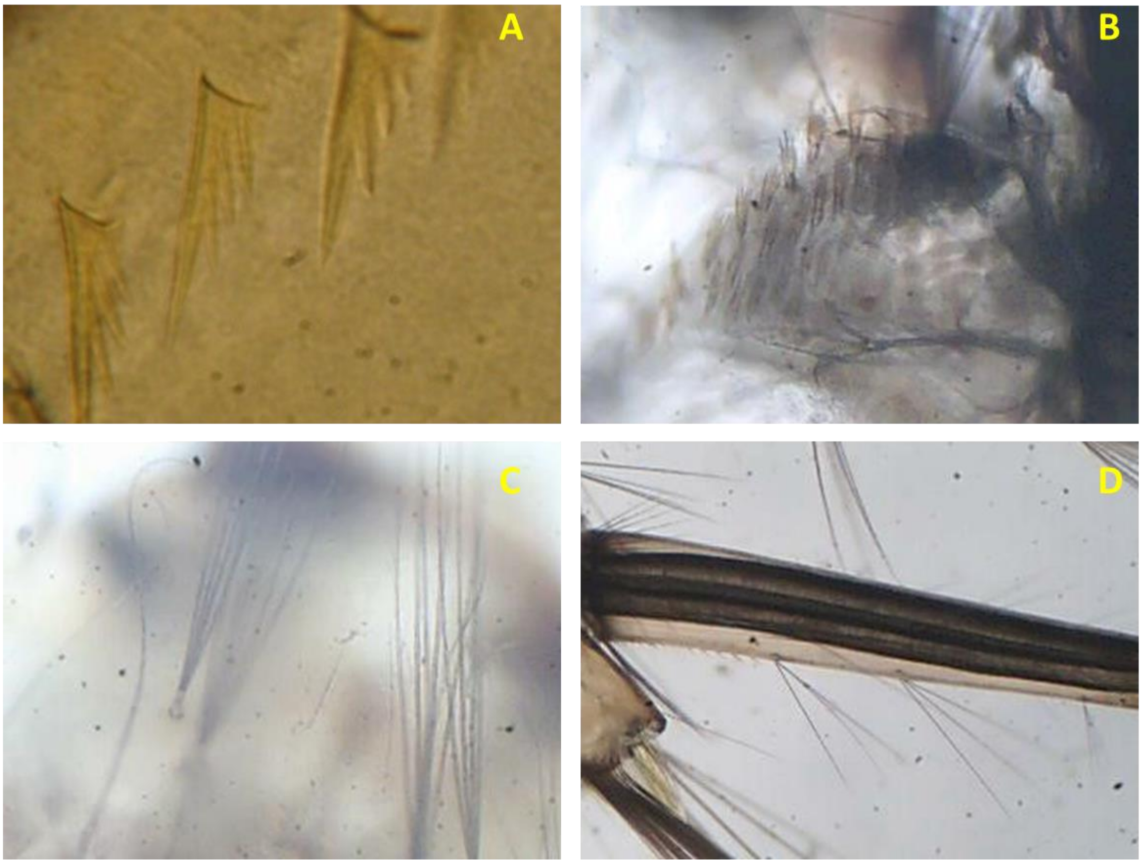


**Figure 21:** Morphological characteristics of identification of *Culex pipiens*

**A:** shape of the distal tooth of the siphon pecten **B:** shape of the comb scales **C:** number of branches in the C setas **D:**general shape of the siphon and number of 1a-S branches

### 1.3.3. *Culiseta longiareolata:*

*Culiseta longiareolata* is a multivoltine with contained development in warm countries and can present a winter diapause, female imagos in cold regions...) Larvae are presented in temperate regions, adults are presented all year round with a maximum of density in spring and another in autumn. females are stenogamous and autogamous. The eggs are united at the time of laying and thus form a pod. The larval sites are of various types (pools, troughs,), the sites are permanent or temporary. The females mainly bite birds, very rarely humans. (SCHAFFNER et al, 2001). In the head of a larva, the antenna is short and the integument of the antenna is smooth (Figure 22.A). The formed siphon: formed only of spine and 1 basal tuft (**Figure. 22.B**).

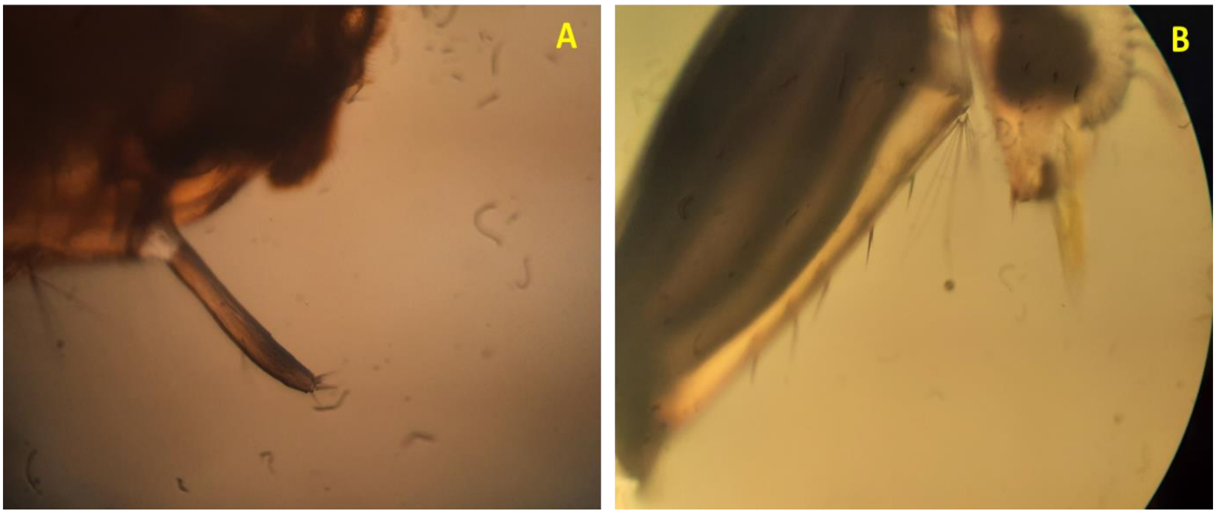


**Figure 22:** Morphological characteristics of identification of *Culiseta longiareolata.*

**A:** smooth antenna **B:** *Cs longiareolata* siphone teft

### 1.3.4. *Anopheles labranchiae*:

*Anopheles labranchiae* is multivoltine and eurygamous (Figure 23). The females, mainly endophilic, are also very anthropophilic. Resting adults can be found in rock crevices or reed hedges. The winter is over and the females lay eggs as soon as the weather is fine.

The larval sites are very varied (ponds, rivers, canals, basins, rice fields, tree hollows, etc.), the water can be fresh or slightly brackish, but always exposed to the sun. Larval development lasts 12 days at 25 C°.

The species has played an important vectorial role in the transmission of malaria in the Mediterranean region and particularly in Italy.



**Figure 23:** Morphological characteristics of identification of *Anopheles labranchiae*

**A:** larvae head seta **B:** *An. labrabnchiae*'s adult wing

### 1.4. Culicidae faunal associations:

Association in animal biology is a difficult concept to demonstrate, particularly as quantitative study methods are not as well developed as in phytosociology. When we began our work on the ecology of mosquito larval populations, we based ourselves solely on the presence or absence of species to try to identify Culicidian associations.

From the results in Table 09, it can be seen that the species *Culex pipiens* can be found with several species, where it can be found in the different breeding sites in the three Wilaya studied. The same observation can be made for *Culiseta longiareolata* which also occupies several habitats of different natures (permanent or temporary, natural or artificial) as well as *An. labranchiae* which frequents mainly natural sites and more or less clear water with other species, we also find *Ae. caspius*. On the other hand, the other species may only have weak associations with other individuals of different species.

**Table 09**: Culicidae faunal associations:(- association absent + : association present).

| Species \ Species | *Aedes caspius* | *Aedes albopictus* | *Aedes geniculatus* | *Anopheles labranchiae* | *Culex pipiens* | *Culex theileri* | *Culex perexiguus* | *Culex hortensis* | *Culiseta longiareolata* |
|---|---|---|---|---|---|---|---|---|---|
| *Aedes caspius* | | - | - | + | + | - | + | - | - |
| *Aedes albopictus* | - | | - | - | + | - | - | - | + |
| *Aedes geniculatus* | - | - | | - | + | - | - | - | - |
| *Anopheles labranchiae* | + | - | - | | - | + | + | - | + |
| *Culex pipiens* | + | + | + | - | | - | - | + | + |
| *Culex theileri* | - | - | - | + | - | | - | - | - |
| *Culex perexiguus* | + | - | - | + | - | - | | - | - |
| *Culex hortensis* | - | - | - | - | + | - | - | | - |
| *Culiseta longiareolata* | - | + | - | + | + | - | - | - | |

### 1.5. Ecological indices:

#### 1.5.1. Ecological composition indices:

**a) The specific or total richness:**

The surveys that were carried out during the five years of the study show variations in the stands sampled. Indeed, we specify the values of the total richness in the four study stations during the surveys carried out (**Table. 10**) in an attempt to highlight the ecological importance of each species in the sampling station.

According to **Table. 10**, the number of species is the highest in the Wilaya of El Tarf with 7 species, while the Wilaya of Guelma presented 6 species. However, the third Annaba there are only urban and temporary sites, a very low number of species was recorded, corresponding to only 5.

**Table 10 :** Total specific richness (S) of the different lodges

| **Wilaya** | Annaba | El Tarf | Guelma |
|---|---|---|---|
| Specific richness | 5 | 7 | 6 |

**b) The richness mean (Sm):**

From **Table 11**, the average richness in the 21 surveyed stations is between 1.5 and 0.63 species. We note that the two stations of Annaba and Guelma present the greatest average richness respectively with values of 1 and 1.5. The El Tarf station presented a richness of about 0.63 species

**Table 11:** The mean richness of the wilaya studied

| **station** | **Annaba** | **El Tarf** | **Guelma** |
|---|---|---|---|
| **Mean richness** | **1** | **0.63** | **1.5** |

**c) Relative abundances or centesimal frequency:**

The numbers and relative abundances of Culicidae for each species and in the three stations are listed in **Table 12**, which shows that the Wilaya of Annaba stands out with the highest number of individuals inventoried (1407 individuals), followed by the Wilaya of El Tarf with 569 individuals, then the Wilaya of Guelma with 272 individuals.

From the graphical representations in figures 24, 25 and 26 which present the relative abundances of all Culicidian species in the three study stations, it can be seen that the three species of *Culex pipiens* , *Culiseta longiareolata* and *Aedes albopictus* are the most abundant species in all sites with intermittent numbers. These results show that relative abundances vary from one station to another and from one species to another

**Table 12:** Numerical importance of the species harvested

| | espèce | N Ind | R.A |
|---|---|---|---|
| **1** | *Aedes caspius* | 4 | 0.178 |
| **2** | *Aedes geniculatus* | 5 | 0.222 |
| **3** | *Aedes albopictus* | 745 | 33.141 |
| **4** | *Anopheles labranchiae* | 43 | 1.913 |
| **5** | *Culex pipiens* | 936 | 41.637 |
| **6** | *Culex theileri* | 17 | 0.756 |
| **7** | *Culex perexiguus* | 23 | 1.023 |
| **8** | *Culex hortensis* | 13 | 0.578 |
| **9** | *Culiseta longiareolata* | 462 | 20.552 |
| **Total** | | **2248** | **100%** |

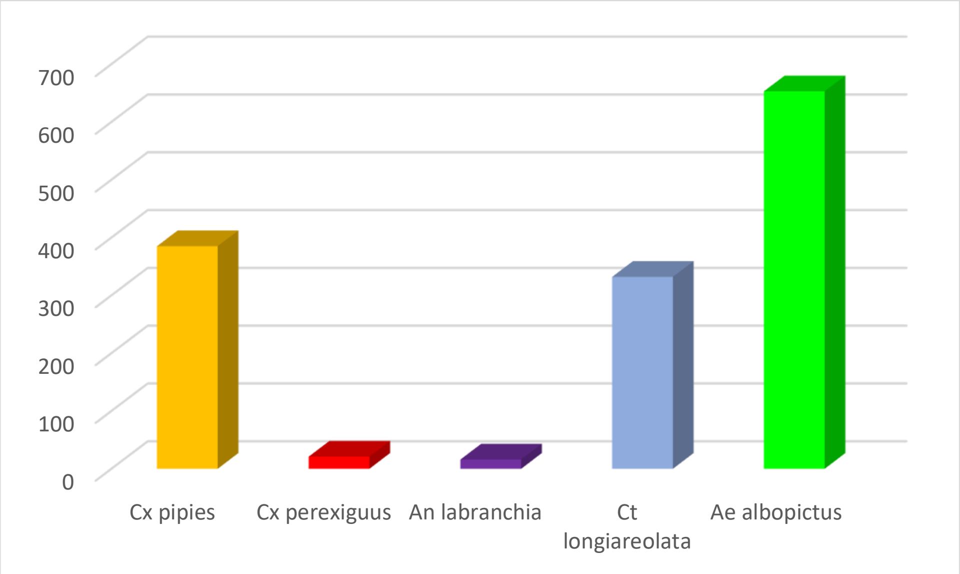


**Figure 24:** The species found in the Wilaya of Annaba.

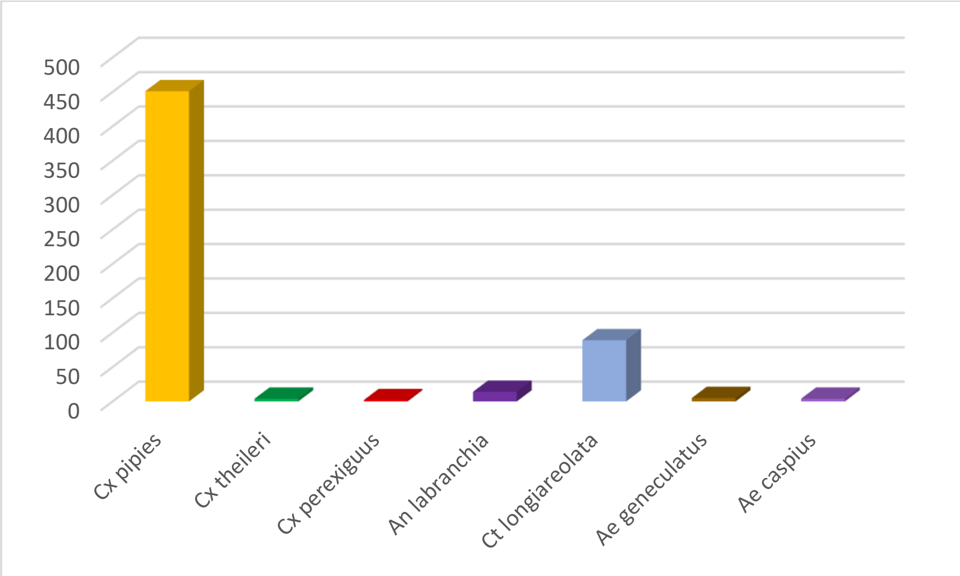


**Figure 25**: The species found in the Wilaya of El Tarf.

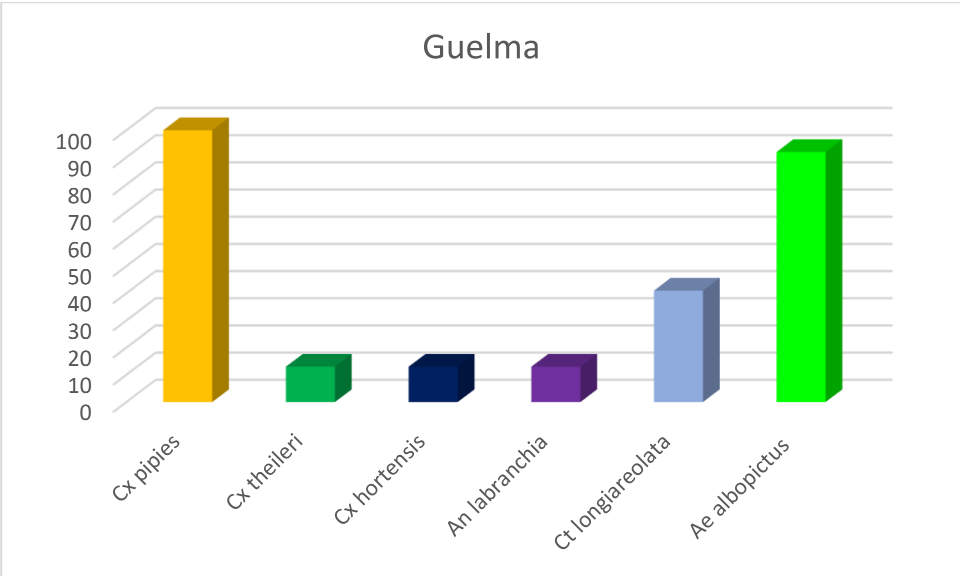


**Figure 26**: The species found in the Wilaya of Guelma.

he observation of the previous figures showed that in the Wilaya of Annaba, the most abundant species are *Culex pipiens*, *Aedes albopictus* and *Culiseta longiareolata* with values respectively of 27.36 %, 46.41 % and 23 %.

For the Wilaya of El Tarf, the most abundant species are *Culex pipiens* (79.26%), and *Culiseta longiarelata* with 15.64%.

The Wilaya of Guelma presented three species as abundant, it is the case of Culex pipiens with 36.76 % Aedes albopictus with 33.92 and Culiseta longiareolata with 15.07%.

### 1.5.2. Ecological Structure Indices:

It is the index that measures the species composition according to their relative abundance, their calculation allows to compare the faunas of two environments when the numbers of specimens collected are very different (Dajoz, 1971).

If we consider the index of (H') by Wilaya, El Tarf gives the highest value 1.45 while Annaba and Guelma are successively 0.76 and 0.7 **(Table. 13)**.

This result indicates that the Culicidae population of the Wilaya of El Tarf is the most diversified with an equitability of 0.81. For the diversity index we can say that Annaba and Guelma are less diversified than El Tarf.

**Table 13:** Ecological structure indices in the three Wilayas.

| Sites / Parameters | Annaba | El Tarf | Guelma |
|---|---|---|---|
| Shannon & Weaver indice **(H')** | **0.76** | **1.46** | **0.7** |
| Equitability **(E)** | **0,47** | **0.81** | **0,36** |
| Concentration **(C)** | **0.35** | **0.28** | **0.65** |
| Specific diversity **(D)** | **0.65** | **0.72** | **0.35** |

## 2. Ovitraps inventory results:

### 2.1. Appearance and distribution of *Aedes albopictus* in Annaba

In the summer 2017, during a mosquito inventory, we trapped an adult female of *Aedes albopictus* in the region of Annaba, extreme northeast of Algeria, which was taken to the lab to be identified using the software of the European identification key for mosquitoes, Schaffner *et al*. (2001), this should be considered the first record of the species in the north-east of the country. This was followed by several complaints from people in the area about a series of mosquito bites causing infections, assuming that it was the Asian tiger mosquito.

### 2.2. *Aedes albopictus* samples collected:

After the initial sighting of *Aedes albopictus* that occurred in 2017 (only one adult female), the research team decided to focus its work on this new species of mosquito that appeared in the region of Annaba. The obtained results show the population development throughout the past four years in the area. **Table 14.** shows the total specimens collected at every site, which then spread to new sites in the following years. We recorded the highest population in Zaafrania (712 eggs, 528 larvae, and 12 adults) in the year 2020. Also, the number of sites infested with *Aedes albopictus* had increased to nine sites in the region of Annaba.

By the end of 2020, we sampled a total of 1442 eggs, 917 larvae, and 18 adults from 7 infested sites. Also, both El Bouni 1 and El Bouni 2 were constant sites that had been positive with *Aedes albopictus* throughout the years.

**Table 14:** *Aedes albopictus* specimen collected from 2017 to 2020 in the region of Annaba.

(E : Egg ; L : larva ; A : Adult)

| Sites | GPS coordinats | 2017 | | | 2018 | | | 2019 | | | 2020 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | E | L | A | E | L | A | E | L | A | E | L | A |
| **El Bouni 1** | 36°50,977'N 007°44,687'E | | | 1 | 241 | 211 | 8 | 17 | 34 | | 132 | 61 | 2 |
| **El Bouni 2** | 36°50,872'N 007°44,550'E | | | | 96 | 79 | | 322 | 194 | 5 | 279 | 87 | 1 |
| **Oued forcha** | 36°54,548'N 007°44,096'E | | | | | | | 520 | 246 | | | | |
| **El-rym** | 36°52,837'N 007°43,659'E | | | | | | 1 | | | | | | |
| **Oued dhab** | 36°53,522'N 007°44,516'E | | | | | | | | | | | 40 | |
| **Bouhdid** | 36°53,299'N 007°42,682'E | | | | | | | | | | | 6 | |
| **L'orangerie** | 36°54,127'N 007°44,558'E | | | | | | | | | | | 26 | |
| **Zaafrania** | 36°45,649'N 007°45,189'E | | | | | | | | | | 712 | 528 | 12 |
| **Elhadjar** | 36°46,805'N 007°43,694'E | | | | | | | | | | 91 | 17 | 2 |
| **Total** | | | | 1 | 337 | 290 | 9 | 859 | 474 | 5 | 1442 | 917 | 18 |

### 2.3. Description of the breeding sites:

*Aedes albopictus* was never found in natural sites. It preferred artificial breeding habitats made of plastic, metal, and clay that come in many forms, such as buckets, pots, tanks, and discarded tires. These breeding sites are also of different sizes; 5, 10, 20, and 200 liters. We have noticed that breeding sites of *Aedes albopictus* are in gardens with a high density of vegetation, specifically under trees and in less windy areas. (**Figure 27**)

**Figure 27:** The different type of artificial breeding sites where we found *Aedes albopictus.*

### 2.4. Culicidae Populations structure collected from the ovitraps:

In the years 2019 and 2020, we noticed an increase in *Aedes albopictus* populations in the area where they started to cohabit with *Culex pipiens* and *Culicita longiareolata*. Therefore, we wanted to know the status of each of these species by following the ovitraps method and also to know if *Aedes albopictus* had reached the surrounding areas of Annaba (Guelma and El Tarf). So, we chose three points for the ovitraps.

The results of the total individuals collected during the whole study period of each species (*Culex pipiens*, *Culiseta longiareolata*, and *Aedes albopictus*) represented in **Figure 28**. We sampled 6798. Among all collections, 1109 (16,31%) specimens were of *Culex pipiens*; 2069 (30,44%) belonged to *Culiseta longiareolata;* and 3620 (53,25%) were of *Aedes albopictus*.

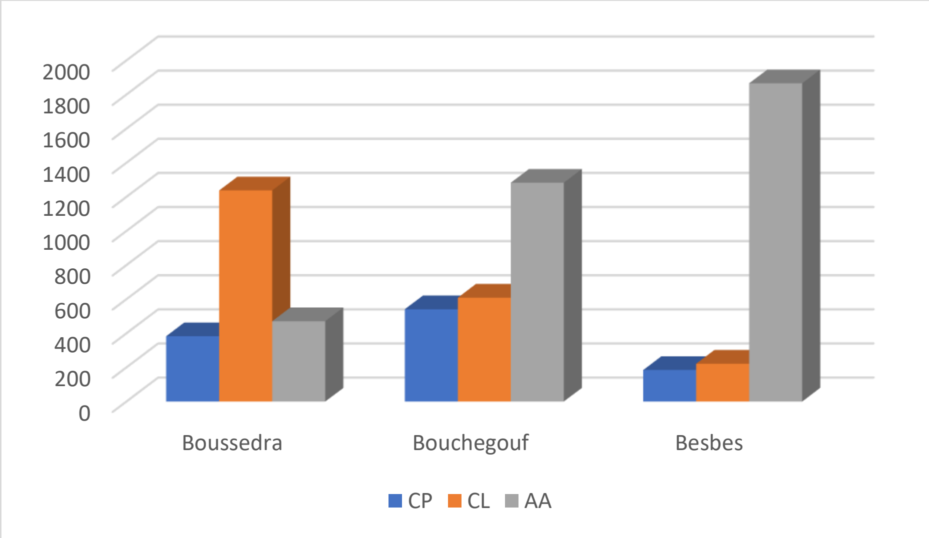


**Figure 28:** The highest record of each species according to the sampling sites.

(CP: *Cules pipiens*, CL: *Culiseta longiareolata*, and AA: *Aedes albopictus*)

**Table. 15** represents the total samples of each species from different sites. Roughly over half of the samples collected from El Bouni belong to *Culiseta longiareolata* (59,18%), whereas 22,51% were *Aedes albopictus*, and 18,31% belong to *Culex pipiens*. In Bouchegouf, we obtained 52,73% of *Aedes albopictus*, with 25,03% of *Culiseta longiareolata* and 22,24% of *Culex pipiens*. While Besbes' simples revealed 82,09% *Aedes albopictus* and only 9,77% and 8,14% *Culisita longiareolata* and *Culex pipiens*, respectively, These results indicated that Besbes didn't only have the highest record of *Aedes albopictus* simples but also the highest record of all three species.

**Table 15.** Number, percentages and maximum density of species collected in the three sites.

(CP: *Cules pipiens*, CL: *Culiseta longiareolata*, and AA: *Aedes albopictus*)

| | **CP** | | | **CL** | | | **AA** | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number | % per site | Max density | Number | % per site | Max density | Number | % per site | Max density |
| El Bouni | 383 | 18,31 | 21,8 | 1238 | 59,18 | 41,5 | 471 | 22,51 | 14,1 |
| Bouchegouf | 541 | 22,24 | 18,5 | 609 | 25,03 | 15,9 | 1283 | 52,73 | 41,5 |
| Besbes | 185 | 8,14 | 7,3 | 222 | 9,77 | 12,4 | 1866 | 82,09 | 45,9 |
| **Total** | 1109 | | | 2069 | | | 3620 | | |
| **Mean** | | | 15,87 | | | 23,27 | | | 33,83 |
| **% per species** | | 16,31 | | | 30,44 | | | 53,25 | |

The average number of larvae of *Culex pipiens* was the highest in Bouchegouf (22,542 ± 48,992).as well as the average of *Culiseta longeariolata* in El Bouni (51,583 ± 104,678) while the average of *Aedes albopictus* was the highest in Besbes (77,75 ± 127,108).

The data analysis of species in all sites, in which *Culex pipiens* ($F_{2,69}$ = 0,74; P = 0,481), *Culiseta longeariolata* ($F_{2,69}$ = 0,59; P = 0,556), and *Aedes albopictus* ($F_{2,69}$ = 1,24; P = 0,296) showed no significant difference between the average number of larvae of each species in the three sites.

On the other hand, the average number of larvae in each site where we have El Bouni and Bouchegouf ($F_{2,69}$ = 0,41; P = 0,665), ($F_{2,69}$ = 0,71; P = 0,495) successfully with no significant difference between the species in both sites, but in Besbes ($F_{2,69}$ = 7,14 ; P = 0,002) the average difference between the species was highly significant **(Table 16)**.

**Table 16.** Number of larvae of each species (CP, CL, and AA) in each site (B, F, and S).

Species: (CP : *Culex pipiens*, CL : *Culiseta longeariolata*, AA : *Aedes albopictus*)

Sites : (B : Boussedra F : Bouchegouf S : Besnes)

| | **n : average ± Ecart type (Min-Max)** | | | **Kuskal-Wallis** |
|---|---|---|---|---|
| | **B** | **F** | **S** | |
| **CP** | 15,95±49,735 (0-218) | 22,542±48,992 (0-185) | 7,708±21,282 (0-73) | $F_{2,69}$= 0,74 ; P = 0,481 |
| **CL** | 51,583±104,678 (0-415) | 25,375±45,282 (0-159) | 9,25±27,173 (0-124) | $F_{2,69}$= 0,59 ; P= 0,556 |
| **AA** | 19,625±41,114 (0-146) | 53,458 ±111,839 (0-415) | 77,75 ±127,108 (0-459) | $F_{2,69}$= 1,24 ; P= 0,296 |
| **Kuskal-Wallis** | $F_{2,69}$= 0,41 ; P= 0,665 | $F_{2,69}$= 0,71 ; P= 0,495 | $F_{2,69}$= 7,14 ; P= 0,002 | - |

In all three surveys, the larvae's presence was from April to October, then it almost completely disappeared from November to March. **Figure 29** shows the development of the population of the three species throughout the time in each site (A, B, and C) and also the total of all sites (D).

In El Bouni, *Culex pipiens* (218 specimens in early May) and *Culiseta logiareolata* (415 larvae in late May) established the site from early April to July. Moreover, *Aedes albopictus* appears at the site later (late July) after almost both Culex pipiens and Culiseta longiareolata completely disappear, leaving the site for itself till late November (459 larvae in late August). In Bouchegouf also, *Culex pipiens* (185 early May) and *Culiseta longiareolata* (159 early July) shared the site from April to July, but only late June *Aedes albopictus* came along till late July to have its peak later (415 early August) and disappear in November. In Besbes, both *Culex pipiens* and *Culiseta longiareolata* had a shorter period on the site (from April to early June) and once *Aedes albopictus* was found, it had the site till early November (459 late August).

The D graph demonstrates the number of mosquito larvae of all species at the three sites combined, where we can see clearly that the presence in large numbers was for *Culex pipiens* in the early season, for *Culiseta longiareolata* at mid-season and by the end of the season it was only *Aedes albopictus,* even though they all shared what we call the transition period.

The specimens collected showed that *Aedes albopictus* is capable of sharing breeding sites with several species (especially from June to July), even at times when we found all three of them together. Then it dominates the sites for months, more specifically during summer (the dry and hot period).

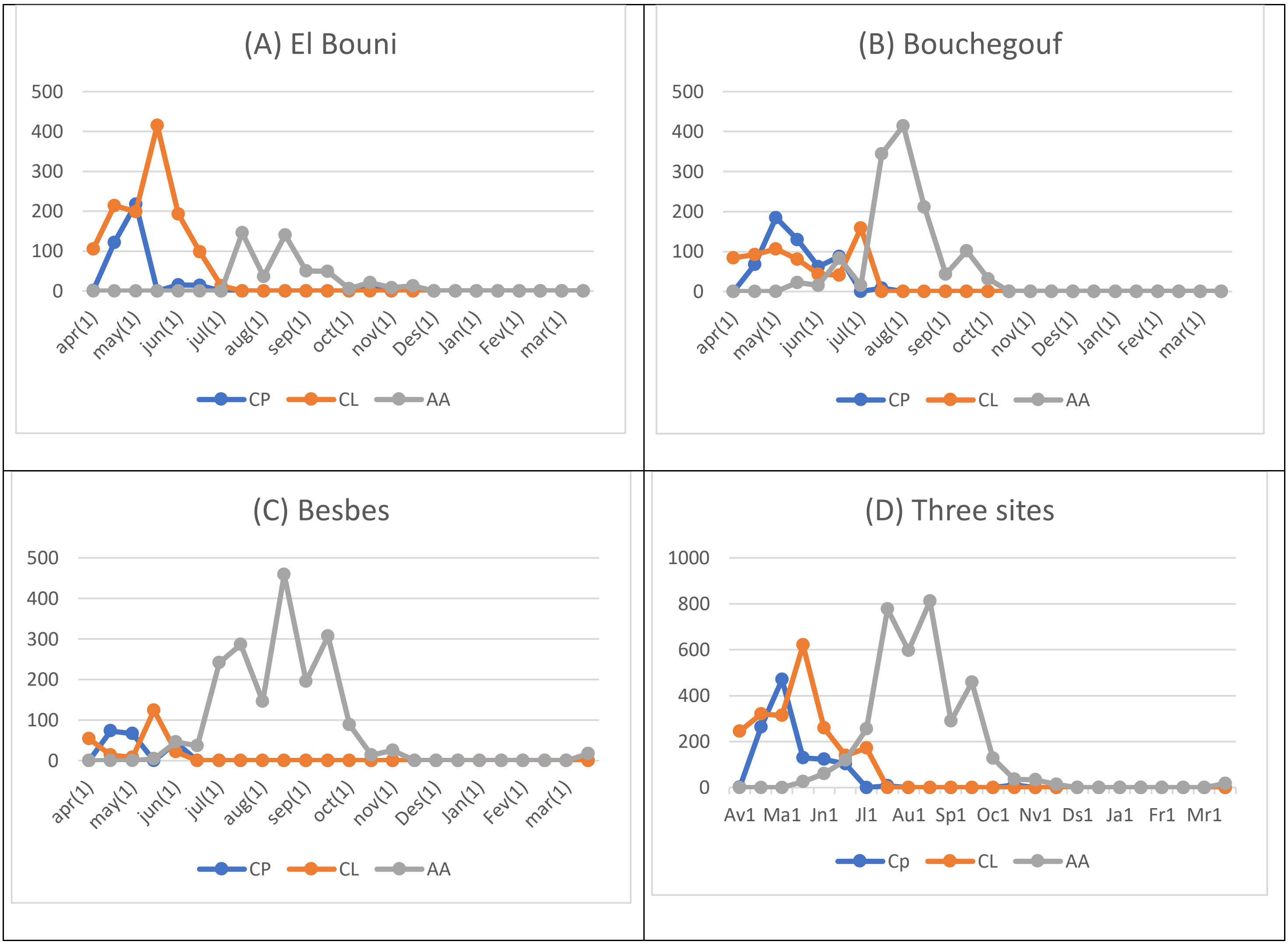


**Figure 29.** Number of mosquito larvae collected every two weeks from study site in El Bouni, Bouchegouf; Besbes (April 2021- March 2022).

(CP : *Culex pipiens*, CL : *Culiseta longiareolata*, AA : *Aedes albopictus*)

## 3. Toxicological study:

To determine the toxicological activity of the insecticide product Spinosad we applied doses (200, 100 and 50µg/L) to newly exuviated Culex pipiens larvae (L4) for 24, 48 and 72 hours.

### 3.1. Study of the toxicity of Spinosad on *Culex pipiens* larvae after 24, 48, and 72 hours*:*

The mortality rates of *Culex pipiens* larvae recorded in the means of 3 replicates after 24, 48, 72 hours observe and correct for the different concentrations are represented in (**Table 17**) as well as the F observe and P error. An increase in mortality rates is observed with varying means between 20% (50µg/l) and 70% (200µg/l) after 24H with a dose response relationship which shows that Spinosad has a larvicidal effect on *Culex pipiens* larvae. Which increases with the duration of exposure.

**Table 17:** Analysis of variance of means of mortality of *Culex pipiens* larvae exposed to Spinosad after 24, 48 and 72 hours

(O.M : Observed mortality C.M: Corrected mortality).

| | **50** | | **100** | | **200** | | **Control** | **$F_{obs}$** | ***P*** |
|---|---|---|---|---|---|---|---|---|---|
| | O.M | C.M | O.M | C.M | O.M | C.M | | | |
| **24H** | 23.33 | 23.33 | 38.33 | 38.33 | 70 | 70 | 0 | 31.26 | 0,113 |
| **48H** | 66.67 | 66.05 | 70 | 69.12 | 88.33 | 87.98 | 1.67 | 4.904 | 0,27 |
| **72H** | 76.67 | 76.27 | 83.33 | 83.05 | 95 | 94.92 | 1.67 | 12.131 | 0,178 |

After an angular transformation of the mortality percentages, the data were subjected to an analysis of variance at a classification criterion which shows that at the significance level $\alpha = 0.05$ the null hypothesis of equality of variances cannot be rejected, and this after the observation of the variances of the $F_{obs}$ (31.26, 4.904 and 12.131) and for the *P*-value (0.113, 0.27 and 0.178) after 24, 48 and 72 hours respectively.

The regression line (**Figure 30**) is made by the probits of the percentage corrected mortality expressed as a function of the decimal logarithm of the doses. The coefficient of determination $R^2 = 0.969$, 0.831 and 0.924 for 24, 48 and 72 hours of treatment successively reveals a positive relationship between the probits and the logarithm of the doses tested.

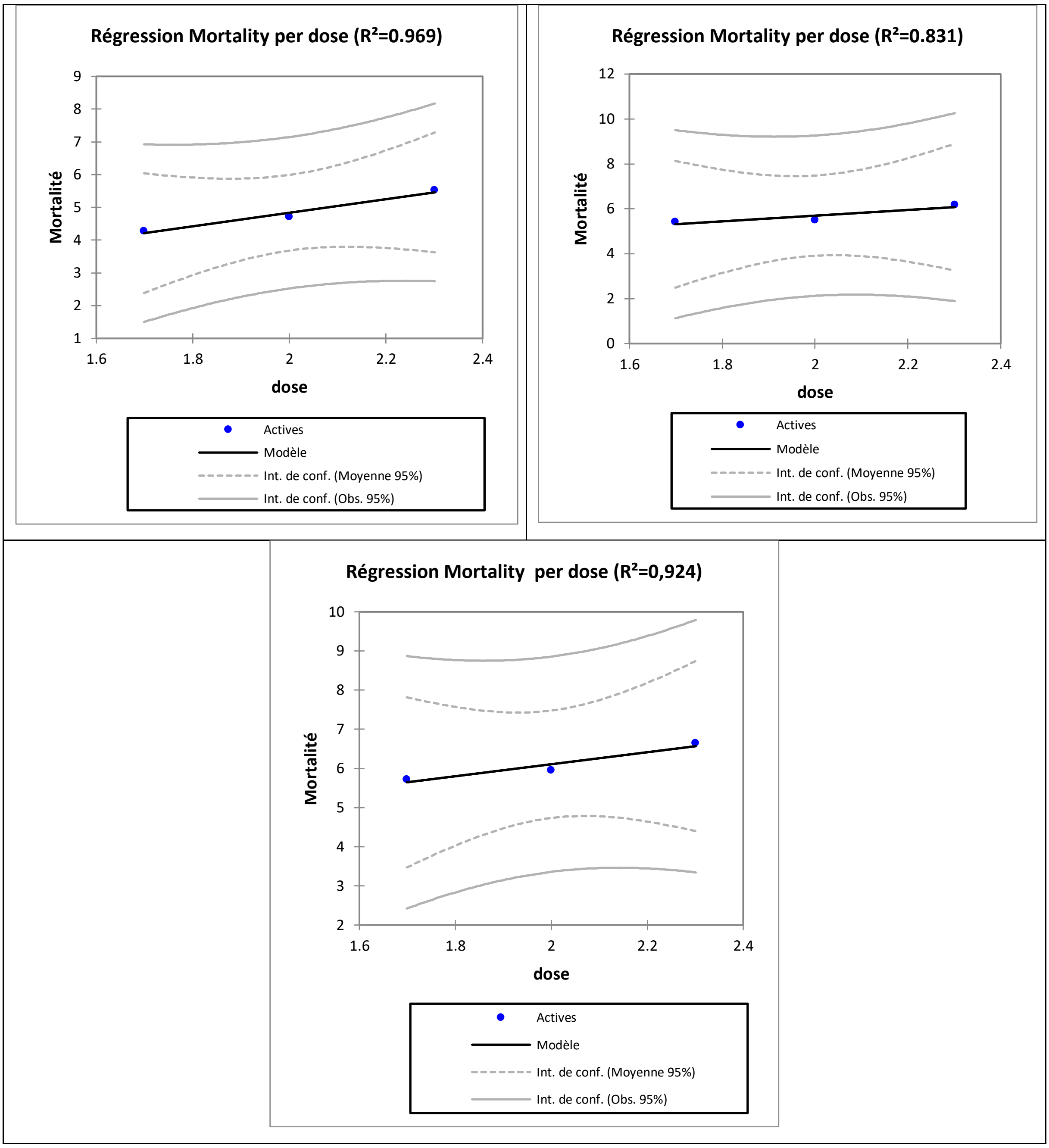


**Figure 30:** Reference curve expressing the probits as a function of the decimal logarithms of the doses in 24, 48, and 72 hours of exposure.

### 3.2. Study of the toxicological parameters of Spinosad on $L_4$ larvae of *Culex pipiens:*

**Table 18:** Toxicological parameters of Spinosad after 3 successive days of exposure.

| Time | Equation | $R^2$ | Slope | LC 50% CI (95%) | LC 90% CI (95%) |
|---|---|---|---|---|---|
| **24 h** | Y = 0.70 +2.07X | 0.969 | 3.02 | 119.47 | 497.30 |
| **48 h** | Y = 3.16 +1.27X | 0.831 | 6.06 | 28.11 | 287.26 |
| **72 h** | Y = 3.04+1.53X | 0.924 | 4.46 | 19.10 | 131.51 |

The results shown in **(Table 18)** summarise the different toxicological parameters of the pesticide Spinosad. These show that there is a strong correlation between the mortality rate and the time of exposure of mosquitoes to different concentrations of insecticide.

The regression line after a one-day larval treatment exposure is of the form Y = 0.70 + 2.07X ($R^2$= 0.918). To ensure 50% mortality of *Culex pipiens* larvae after 24 hours, the concentration of Spinosad must be equal to 119.47 µg/l, whereas 497.30 µg/l is required to ensure 90% mortality of the treated larvae.

48h after treatment, the regression line is given by the formula: Y = 3.16 +1.27X with R2= 0.831 **(Table. 18)**. The results also show that there is a strong correlation between the concentrations and mortality ($R^2$ = 0.831) and gives a mortality of 50% and 90% at the doses 28.11 µg/l and 287.26 µg/l when arriving at the third day, the linear regression of which is given by the form Y = 3.04+1.53X, the Cl 50 and Cl90 are 19.10 and 131.51 µg/l successively.

According to the values given in **(Table 18)**, it can be seen that the pesticide used has a larvicidal effect on *Culex pipiens* larvae. The $R^2$ equals 1 so the probability that the equations provided correctly reflect the relationship between the data is good.

# III. Discussion

**1. Taxonomic study:**

Our study region is characterized by a strategic and interesting geographical location in the Mediterranean basin. The diversity of ecosystems and the presence of several wetlands help to have an interesting biodiversity. The presence and wide distribution of mosquitoes in our study area remains an important and necessary subject to be taken into consideration because of the influence of this population on these ecosystems and public health due to their nuisance and considered as potential pathogenic vectors.

The inventories were carried out in 21 different sites scattered in the three Wilayas. The field surveys allowed the identification of 9 species of Culicidae belonging to 4 genera (*Culex*, *Culiseta*, *Aedes* and *Anopheles*). The genus *Culex* is mainly represented by the species *Cx. pipiens* and in second position *Cx. theileri*, *Cx. hortensis* and *Cx. perexiguus*. While the genus *Aedes* was represented by three species: *Ae. Albopictus*, *Ae. geniculatus* and *Ae. caspius*, the two genera *Culiseta* and *Anopheles* were represented by only one species *Cs. Longiareolata* and *An. Labranchiae* successively. Based on literature, previous studies on Culicidae have reported the presence of 53 species in the whole Algerian territory **(Merabti *et al* 2021).**

Over a period of thirty years of fieldwork, **Senevet & Andarelli (1960)** recorded a total of 27 species of Culicidae in the Algiers region, belonging to two sub-families, the Anophelinae and the Culicinae. In addition, the inventory of mosquitoes in the interior of the country revealed the presence of 12 and 7, in Mila **(Senevet & Andarelli, 1966)** and Constantine **(Messai *et al*, 2010)** respectively.

The data collected according to the methods we used gave different results, depending on the operator, the technique used, the nature of the site, the place and time of collection, and the accessibility of the site. The analysis of the species composition of Culicidae in the different study sites shows first of all that each environment presents a particular faunistic feature. Indeed, *Cx. pipiens* and *Cs. longiareolata* develop in all types of lodgings, whether artificial or natural. There are common species for artificial sites *Aedes albopictus.*

In addition, several other works mention the species ***Culex pipiens*** such as **(Bendali, 1989)**, **(Hassaine, 2002)**, **(Lounaci, 2003)** who described the species and confirmed its existence in the different stations of Algiers and Tizi-Ouzou. **Berchi (2000)** confirms the existence of this species in the urban and sub-urban environments of Constantine and more particularly in deposits rich in organic material. It is the same for **(Kerboua & Merniz, 1997; Boudrihem, 2001; Lounaci, 2003; Hamaidia, 2004; Bebba, 2004; Merabti, 2016** and **Matoug, 2018)** who found this species in very diverse deposits.

***Culiseta longiareolata*** inhabits a wide range of habitats, and its ecological optimum is reached in clear waters **(Hassaine, 2002; Himmi, 2007)**. Moreover, this species was collected by **(Berner, 1974)** and **(Hassaine, 2002)** in waters rich in organic material. This does not agree with the data found by **(Louah, 1995)**. In Portugal, this species has been found in cement pools for domestic or agricultural use **(Ramos *et al*, 1977/78)**.

This widely distributed species is found in the southern Palearctic, eastern and Afro-tropical regions. It is very common throughout Mediterranean Africa (**Becker *et al,* 2010 ; Becker & Hoffmann, 2011**). Breeding sites are often found in man-made waterbodies. This mosquito species is associated with vegetable gardens, farms, and domestic hen houses (**Möhlmann *et al*. 2017, Toma *et al*. 2020**). However, *Cs. longiareolata* is also found in fresh water rock pools and other waterbodies in natural area (**Schaffner *et al*., 2010, Möhlmann *et al*., 2017**). Adults has been described as a thermophilic and ornithophilic species that rarely bites humans (**Becker *et al*. 2010, Martínez-de la Puente *et al*. 2020**). Cette espèce ne pique pas l'homme et son rôle de vecteur de parasitoses humaines ne peut être que des plus réduits **(Shalaby, 1972 ; Trari B, 2017 ; Hassaine, 2002 ; Rubén and Ricardo, 2011).**

***Ae. Albopictus***, which is a new addition to the Culicidae fauna in north-eastern Algeria (**Aroussi et *al*, 2021 ; Hamaidia & Soltani, 2021 ; Rouibi et *al,* 2023**), was first declared in Tizi Ouzou in 2010 by **Izri *et al* (2011)**, followed by several reports and claims in neighbouring regions: Oran in 2015 (**Benallal *et al*., 2016**) Algiers in 2016 (**Benallal *et al*., 2019**). This species is a vector for dengue, chikungunya, yellow fever, Japanese encephalitis, **Giatropoulos *et al*. (2012**); **Kampen *et al*. (2013)**, and zoonoses, such as dirofilarioses, **Giangaspero *et al*. (2013)**; presenting a notable threat to public health.

***Aedes caspius*** is characterised by a frequency (15.05%) in the Annaba region. It was found in Touggourt **(Boudrihem, 2001)**, in four stations in the eastern region of Algiers, the Réghaia marsh and the Sébaou wadi in Tizi Ouzou **(Lounaci, 2003),** In the oases of the Biskra region (south-east Algeria) **(Benhissen *et al*, 2017),** in the region of M'Zab-Ghardaïa **(Boukraa *et al.*, 2013)**,in Oued Righ **(Bebba, 2004)**, in Tebessa **(Bouabida, 2012),** in deposits that are characterised by fairly high salt contents. **Boulknafet (2006)** reported the presence of this species in seawater with very high salinity. This species shows a remarkable accessibility to temporary salt water levels (rock hollows), has a wide distribution and extends over almost the entire Palaearctic region including Europe, North Africa and Asia north of the Himalayas **(Sinegre, 1974)**. This species is found in many regions. Indeed, it is reported all along the Mediterranean coastline **Seguy (1924)**. It is widespread on all shores of France **(Rioux, 1958)**. In Algeria **Senevet** & **Andarelli (1960)** report the highest frequency in the Oranese. According to **Sinegre (1974)** this halophilic species is particularly fond of waters with low or medium salinity, whereas our collections show that this species is sampled in seawater with very high salinity.

***Culex perexiguus*** is abundant during the summer and autumn. These larvae develop in many types of natural and even artificial habitats **(Trari, 2017)**. **Seguy (1924)** said that this species has the synonyms *Culex decens* (Storey, 1918) and *Culex univittatus* (Theobald, 1901). *Cx. perexiguus* is a vector species for West Nile virus, Sindbis virus and Rift Valley Fever **(Brunhes et *al.*, 1999)**. It frequents fresh water but can grow also in salt water **(Brunhes et *al.*, 2001)**. This species is widespread in North Africa and Southwest Asia, from where its range extends to India. This species is also reported from Italy **(Severini *et al*., 2009)**, in Spain **(Eritja *et al*., 2000; Bueno-Mari, 2012)**, in addition to Albania, Bulgaria, Greece and Portugal **(Snow & Ramsdale, 2004)**. Recently, *Cx. perexiguus* was found in Arabie Saoudite **(Al Ahmad *et al*., 2011)**. In North Africa, Cx. perexiguus is known from Egypt **(Harbach, 1988)**, Mauritania **(Hamon *et al*., 1966)**, of Libya **(Goodwin, 1961)**, Tunisia **(Moussiegt, 1983, Bouattour, 2013)**, Algeria **(Tine-Djebbar *et al*., 2011; Bouabida *et al*., 2012)**, and Morocco **(Trari *et al*., 2002; 2017)**. The larvae of *Cx. perexiguus* develop in a wide variety of water collections (domestic and wild) where the water is generally clean and fresh to slightly brackish. Trophic behavior *Cx. perexiguus* appears to be an exophilic mosquito, but can also, exceptionally, enter houses to bite humans **(Kirkpatrick, 1925)**. Females feed mainly on birds **(Jupp, 2001; Gratt, 2009)**, but they can also bite cattle **(Gad et al., 1999)**.

***Culex hortensis*** has never been implicated in the transmission of human parasitosis. It is known from Morocco, Tunisia but also from Algeria. According to **(Brunhes *et al,* 1999).** The larvae of this species are found in small, unvegetated roosts. It can develop up to an altitude of 3000m. The females do not bite mammals. They do however feed on amphibians and reptiles **(Brunhes et al, 1999).** We sampled this species in June in the commune of Roknia, Wilaya de Guelma, a temporary site with turbid and shallow stagnant water. **Andarelli (1954)** found it in irrigation channels north of Aures, **Lounaci (2003)** collected it in poorly vegetated sites and in the Reghia marsh. **Hamaidia (2004)** collected this species in permanent and temporary deposits with or without vegetation, in the regions of Souk-Ahras and Tébessa. **Boulknafet (2006)** reported its existence in Skikda.

***Culex theileri*** wasn't found in many site during our inventory. This species extends from North Africa to Russia, from Europe and Morocco to India and Nepal **(Brunhes *et al*, 1999).** It is frequent in various deposits, such as polluted deposits, permanent deposits rich in vegetation, and temporary deposits with stagnant water with or without vegetation. It has been found in several regions of Algeria. **Senevet & andarelli (1960)** report the existence of this species in Algiers and Oran. **Berchi (2000)** in Constantine. **Clastrier & Senevet (1961)** report the existence of the species in two regions of the Algerian Sahara, El Golea and Ain Emgeul. **Lounaci (2003)** states that this species prefers natural roosts. **Hamaidia (2004)** encountered it in the regions of Souk- Ahras and Tébessa. **Bebba (2004)** in Oued Righ and **Matoug (2018)** in Skikda and Guelma.

The genus Anopheles which has also been inventoried in our study area is represented by one species : *Anopheles labranchiae.* Base on **Benmansour (1972),** *Anophèles labranchiae* is one of the main species of the Moroccan Anophelian fauna. This species is the only one identified in North Africa **(Guy, 1959 ; Senevet et Andarelli, 1960 ; Brunhes et *al*, 1999)**. The larva of this species was found in residual pools with green vegetation and in the bed of the wadis **(Andarelli, 1954). Brunhes et *al* (1999)** shows that the water in these deposits can be fresh or slightly brackish, but always exposed to the sun.

**Lounaci (2003)** captured this species in the park of the Agronomic Institute of El-Harrach and in Oued Saboun (Tizi-Ouzou). **Berchi (2000)** reports the presence of this species in swamps near Constantine. **Hamaidia (2004 - 2021)** captured this species in Souk-Ahras and Tébessa. **Bebba (2004)** captured this species in Oued-Righ, in permanent and temporary deposits with stagnant water rich or poor in vegetation.

The inventory of the results of the systematics of Culicidae of the region of Tébessa realized by **Bouabida *et al*. (2012)** reveals the presence of 9 species belonging to a single subfamily; that of the Culicinae, where they noted three tribes, the tribe of the Aedini is represented by a single species: Ochlerotatus caspius, the tribe of the Culicini is formed by a single genus, that of the Culex which contains 5 species, these are: *Culex pipiens, Culex theileri*, *Culex hortensis, Culex perexiguus,* and *Culex laticinctus*. The tribe of Culisetini consists of 3 species represented by *Culiseta longiareolata*, *Culiseta annulate,* and *Culiseta subochrea*.

**Arroussi *et al* (2021)** in 2018 made an inventory study in the region of Annaba and determined the properties of larval habitats were recorded for each site. They found 8 species including *Culex pipiens* (Linnaeus, 1758), *Culex modestus* (Ficalbi, 1889), *Culex theileri* (Theobald, 1903), *Culiseta longiareolata* (Macquart, 1838), *Anopheles labranchiae* (Falleroni, 1926), *Anopheles claviger* (Meigen, 1804), *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894). Among the species, *Cx. pipiens* presented the highest species abundance (RA %) (55.23%) followed by *Cs. longiareolata* (20.21%). The Aedes species are recorded for the first time in the study urban area. Variation of diversity in different sites depends on the type of breeding habitat.

## 2. Oviposition study :

*Aedes albopictus* is a recent invasive species in the Mediterranean region **(Adhami et al., 1998)**. Several factors contributed to the rapid spread of this mosquito species. Among the most important are the resistance of its desiccated, dormant eggs and their efficient passive transport around the world, often in used tires **(Tatem *et al*., 2006)** or in imported plants **(Benallal *et al*., 2019)**.

In Algeria, the first observation of this species was in 2010 in the region of Tizi-Ouzou by **Izri *et al.* (2011)** to be the first report for the species in the country. Then it was followed by other observations in other regions of the country, which were the region of Oran in 2015 by **Benallal *et al*. (2016)**; Algiers in 2016 by **Benallal *et al*. (2019)** and **Merabti *et al* (2021)**, the region of Annaba in 2018 by **Arroussi *et al.* (2021)**; and last in 2020 in the region of Souk Ahras by **Hamaidia & Soltani (2021)**. Our research team notes the first observation of this species in the Annaba region in 2017 and in the regions of El Tarf and Guelma in 2021, as recorded in our current work, which confirms that it's now spread all over Northeast Algeria.

In the life of a mosquito, the act of oviposition alone determines the survival or death of the next generation **(Subra, 1971)**. This mosquito used to breed in natural habitats, especially in tree holes, leaf axils, rock pools, and similar sites **(Hawley, 1988)**. *Ae. albopictus* is a generalist who readily adapts to diverse environmental conditions in tropical and temperate regions. (**Rai, 1991)**. Our inventory (2017-2020) results conclude that *Aedes albopictus* is found only in the urban environment. We never come across this species in natural sites, only in artificial ones (buckets, barrels, tanks, cups, tires...) It is confirmed by **Swaddiwudhipong *et al.* (1992); Mellander *et al.* (2015)** that the shortage of water supply during the dry season can increase the number of storage containers in the community, plus the discarded containers and solid wastes filled with rainwater can create more breeding habitats because *Aedes albopictus* prefers artificial sites in urban areas. **Gubler *et al*. (2001); Delatte *et al*. (2008)** have interpreted this phenomenon as widespread deforestation, climate change, and an increase in global trade have forced this mosquito worldwide to adapt to breeding in artificial container habitats.

Ovitraps provide useful data on the spatio-temporal distribution of mosquitoes because this monitoring allows one to check the presence and density of the vector at a local scale; **Fisher *et al.* (2017); Nunes *et al.* (2011)**. Although previous inventories of mosquitoes in the area confirm the presence of 18 species of mosquitoes **(Houmani *et al*. (2017), Benmalek *et al*. (2018), and Arroussi *et al*. (2021))**, out of fifty-three species prospered in Algeria from 1903 to 2021 according to **Merabti *et al*. (2021)**, our ovitraps attract only three species (*Culex pipiens*, *Culiseta longiareolata*, and *Aedes albopictus*).

Our results indicate that the resident species of *Aedes albopictus* are *Culex pipiens* and *Culiseta longiareolata*, whereas in other works like those of **Simard *et al.* (2005), Kamgang *et al* (2010), and Kamgang *et al.* (2013)**, they indicate *Aedes aegypti* as a resident species. This is the first study in which there was a comparison between *Aedes albopictus* with *Culex pipiens* and *Culiseta longeariolata*.

*Culex pipiens* was the most abundant species based on most survey studies in Algeria, like **Hamaidia & Berchi (2018)**. Contrary to what is happening now, after the arrival of *Aedes albopictus* in the area, we can see that this last is the most abandoned. Moreover, *Culex pipiens* and *Culiseta longiareolata* showed, in previous studies by **Benhissen *et al.* (2017) and Asloum *et al.* (2021)**, high ecological plasticity and habitat coexistence. Now that behavior is being affected by the appearance of *Aedes albopictus* in the region. A study in the Central African Republic showed that the introduced species *Ae. albopictus* predominated over *Ae. aegypti*, according to **Kamgang *et al.* (2010)**. The coincident decline in several *Culex pipiens* and *Culiseta longeariolata* populations that occurred after *Ae. albopictus* invasions may have resulted from competitive displacement, which gave it an advantage over local species, as mentioned by **Livdahl (1993); Braks *et al.* (2004)** in the competition between *Aedes aegypti* and *Aedes albopictus*, where this last invaded Rio de Janeiro (Brazil). Where competition is most intense, *Aedes aegypti* is eliminated **(Juliano., 1998)**; this is what we see in Besbes (El Tarf), where *Aedes albopictus* is dominant on indigenous species *Culex pipiens* and *Culiseta longiareolata*. Therefore, it is an invasive and adaptive species, coexisting with local mosquito species that could even displace them.

According to the results obtained, *Ae. albopictus* will likely continue to spread to the rest of the country. **Vidal *et al.* (2012)** considered its control difficult due to its rapid reproduction and ability to adapt to different environments.

### 3. Toxicological study:

Of the many groups of biting insects, the Culicidae are probably the best known and most feared both for the parasites they can inoculate during their blood meal, and for the annoyance and nuisance their presence constitutes. The only way to deal with this type of disease is to control the mosquito vector by several methods (chemical, genetic, ecological and biological).

The most effective approach to control is based on the sufficient reduction of mosquito larval populations. This control must be adapted to the knowledge of their breeding sites, their behaviour and their ecology, and is therefore fundamental to ensure the effectiveness of this action **(Djogbénou, 2009)**.

*Culex pipiens* is one of the main vectors of St Louis encephalitis in the United States and has also been considered the main vector of West Nile virus in Romania **(Savage *et al*.,1999),** the United States **(Plamisano *et al*., 2005),** in Bulgaria and Czech Republic **(Hubalek & Halouzka,2002).** Marocco was affected in 1996 **(Harrack & Guenno, 1997)** and 2003 **(Schuffenecker *et al*.,2005**). This species is involved in amplifying the transmission cycle of the virus to birds **(Tardif *et al*., 2003)**. It also acts as a vector for other pathogens such as malaria, yellow fever, dengue fever, filariasis **(Hamon et Mouchet, 1967)** and certain encephalitis **(EL Kady *et al*., 2008).**

In Algeria, *Culex pipiens* is the mosquito of greatest interest because of its abundance and its real nuisance in urban areas **(Berchi *et al*., 2012).** Furthermore, this species is suspected to be involved in West Nile and Rift Valley Fever transmission, according to this context, we define the importance of *Cx. pipiens* as a vector of both viruses in the Maghreb region **(Amraoui, 2012).**

Despite the existence of efficient sustainable alternatives, the agriculture industry is still using organic chemical pesticides for pest control (**Athanassiou *et al*., 2017**). Undoubtedly, the integrated pest management used in agriculture with a traditional approach seems to be insufficient, since these practices involve the indiscriminate application of pesticides, which might have many losses due to leaching, deposition, hydrolysis, photodegradation and microbial activity (**Nuruzzaman *et al*., 2016**). Also, due to the inherent toxicity of pesticides, the health of animals, humans and the entire environment is at risk (**Prasad *et al*., 2017**). On the other hand, various vector species have developed resistance to synthetic pesticides (**Tilman *et al*., 1988**).

Spinosad which results from the fermentation of an actinomycete (Saccharopolyspora spinosa) and acts as a neurotoxin with low environmental and toxicological risk (**Williams *et al*., 2003**). Degradation under field conditions, mainly due to its poor photostability (**Cleveland *et al*., 2002**), has led to its encapsulation using biodegradable polymeric particles (**Huang *et al*., 2017; Dong *et al*., 2019**)

Spinosad toxicity results show a decrease in fecundity and fertility by reducing the number of offspring (eggs, larvae, pupae and adults) of the F1 generation from pesticide-treated "parents"; this decrease in offspring shows a dose response relationship. This impact of spinosad reduces the fecundity and fertility of different insects **(Yin *et al*., 2008 ; Wang *et al*., 2013 ; Maïza *et al*., 2013).**

In larvae, similar effects were reported in *S. littoralis* larvae treated with azaderachtine, and in Anticarsiagemmatalis **(De Almeida *et al*., 2014).** These abnormalities and the inhibition of metamorphosis can be attributed to disturbances in the synthesis and release of the moulting hormone under the action of Spinosad. This effect may explain the mortality of larvae and pupae, because the increase in mortality coincides with the metamorphosis period **(De Almeida *et al*., 2014)**, since the intense metabolic changes are related to the different hormonal systems and under neuroendocrine control **(Gade *et al*., 1997).**

In adults, these observations are in agreement with earlier observations described by **Kirst (2010)**. For Spinosad, these symptoms can be explained by its action via binding to insect's nAChRs **(Wing *et al*., 1998).**

Spinosad has a relatively low toxicity against *T.absoluta*. In contrast, other studies have shown an LC50 of 0.08 to 0.26 ppm in $L_2$ of *T. absoluta* larvae by the IRAC method (leaf dipping) for spinosad **(Roditakis *et al*., 2012**). However, our results are in agreement with those of **Sabbour& Abdel-Rahman (2013),** which showed similar LC50s in other maize lepidopteran pests Ostrinianubilalis, Chilo Agamemnon and Sesamiacretica with LC50s of 166, 179 and 185 ppm respectively. In other lepidopterans, studies have shown that Spinosad causes significant mortality in Lymantriadispar with an LC50 of 8.7 ppm **(Wanner *et al*., 2002),** highly toxic in Spodopteraexigua with an LC50 of 0.293 ppm **(Wang et al., 2013)**; is highly toxic to Helicoverpaarmigera (Hübner) with an LC50 of 0.41 ppm **(Wang *et al*., 2013).** In Diptera, the fly *Glossina palpalis gambiensis Vanderplank a* was found to be very sensitive to Spinosad (LC50 = 2.2 ppm) compared to deltamethrin (4.2 ppm) **(De Deken *et al*., 2004).** Spinosad was also effective against Aedes albopictus with an LC50 of 0.3 ppm **(Bond *et al*., 2004).**

In our work the toxicological parameters show that *Culex pipiens* requires 119.47mg/l of Spinosad to kill 50% of the treated individuals during 24 hours and this concentration decreases in time: 28.11mg/l in 48 hours and 19.10 mg/l in 72 hours. Our results show a difference in the concentration required for the control of *Cx. pipiens* larvae compared to the results of previous studies either on insects in general or even on mosquito species using lower doses which raises the question whether the species studied, *Cx. pipiens*, has some resistance to this product.

# Conclusion

This work was devoted to an ecological and toxicological behavioral studies, where the first part was devoted to getting an idea about the Culicidian population in the North East of Algeria, plus we focused our work on the new addition to the Culicidae fauna in our region, *Aedes albopistus*, and its influence and impact on the native fauna. Subsequently, toxicological tests were carried out on *Culex pipies* larvae using Spinosad and an aqueous extract.

The inventory that took place in sites from Annaba El Tarf and Guelma got us to find 9 species belonging to four genera (Culex, Culiseta Aedes and Anopheles) that are *Aedes caspius, Aedes geniculatus, Aedes albopictus*, *Culex pipiens, Culex hortensis, Culex theileri, Culex perexiguus*. *Culiseta longiareolata*, and *Anopheles labranchiae*. The most abundant species that as known *Cules pipiens* still hold its place but the ovitraps showed that it has got a competition and a very adaptive and resisting species added recently to the mosquitoes of our country which is Aedes albopictus.

After revealing the arrival of the Asian tiger mosquito to the region of Annaba in 2017, we also declared it for the first time in both Bouchegouf (Guelma) and Besbes (El Tarf), to confirm that most of Northeast Algeria is infested with *Aedes albopictus*. We determined that this species prefers urban artificial breeding sites. In Besbes (El Tarf), the ovitraps revealed *Aedes albopictus* dominance over native species *Culex pipiens* and *Culiseta longiareolata* (82,09 %) ($F_{2,69}$= 7,14 ; *P*= 0,002). Thus, this invasive and adaptive species could coexist with local mosquito species, it can even displace them.

The toxicological tests targeting the larvae of *Culex pipiens* using Spinoad we determined after several tries the doses needed (200µg / l ; 100µg / l ; 50 µg / l) showed that the insecticide used has a high efficiency on L4 larvae of the species treated and helped us determine the LC50 and LC90 to be 119.47 and 497.3 µg / l successfully. Mosquitoes are dangerous species that causes the death of millions both people and animals every year, so having control programs that are both affective and ecofriendly is priority.

We have come across few understandings that mosquitoes behaviors are influenced by human activities and climatic changes to acknowledge the fact that we have a hand in the massive growth of these species and the arboviruses evolution. Its important to take actions in the water storage strategies and take cushions that some of these species are highly attracted to artificial breeding sites, especially *Aedes albopictus*.

For future studies we suggest:

- Culicidae species association must have ecological and behavior reasons that we could investigate about so we need an understanding of the type of the Physical-chemical characteristics of Culicidae breeding sites for a better understanding of these species' distribution and habitat selection.
- Take the control strategies to another level and have an idea about more biological control agents such as mosquitoes predators.
- Do not underestimate these species capacities of resistance and try to have an idea about their tolerance range of ecological factors.
- have more studies about Aedes albopictus especially in the medical field due to its ability to transfer several viruses to both human and animals and take into consideration that this species doesn't belong in our region that mean it could become a vector of local viruses.

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## ***International communications:***

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