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Effects of physicochemical and microbiological properties water on breeding sites of mosquitos

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INTRODUCTION

Introdiction

More than 3,500 species of mosquitoes are recorded across the globe ocean (Normandeau-Guimond, 2022), mosquitoes have a necessary role in ecosystems but above all in human and animal epidemiology because as they are the source of food for umerous animals such as birds ,fish, amphibians and insect ; a number of diseases such as filariasis, dengue fever, yellow fever and malaria are carried by mosquitoes As a result of this they used in scientific research to study diseases and viruses and develop vaccines components of mosquito saliva are also used in some medication ,such as local anesthetics. These arthropods occupy an important place in terrestrial fauna as well as aquatic fauna and in the fight against diseases transmitted by their bites (Aouinty *et al.*, 2017).

Mosquitoes are arthropods belonging to the class insects in the animal kingdom. They form the suborder Nematocera in the order Diptera to the Culicidae family (Merabti, 2016)They therefore represent a real public health problem in the majority of countries around the world (Messai *et al.*, 2016). The mosquito's body consists of the head, thorax and abdomen, where the head contains the eyes, claws and mouth; the thorax contains the wings and legs; and the abdomen contains the digestive, breathing and reproductive systems.

The abundance and distribution of mosquitoes are closely linked to larval breeding sites that are favorable for their reproduction (Hery *et al.*, 2021), they live and reproduce in stagnant water, especially stagnant water, but their development and reproduction are affected by the physicochemical factors of water, such as : temperature ,pH , turbidity, conductivity, chloride, nitrate, total hardness, demand oxygen and biological oxygen demand.....microbiological factors also have an impact on its development.

In addition to physicochemical parameters, bacteriological features of water also have a significant influence on the modulation of the oviposition behavior of gravid female mosquitoes (Seal and Chatterjee, 2023), thus making it more or less suitable for the survival of different mosquito species. These breeding habitat bacteria serve as a direct food source for mosquito larvae (Trexler *et al.*, 1998).

To this end, we analyzed their habitat by the determination of ecosystem surface water physicochemical parameters (Messai *et al.*, 2016) and Knowledge on where mosquitoes breed and why they prefer certain water bodies over others is very important for sound mosquito control strategies.

The main objective of our study is to better understand the effect of these physicochemical and microbiological properties on the larval breeding sites and its distribution in order to be able to control these mosquitoes.

In the present study, we attempted to study this theme like the work done on the same theme the effect of the physicalchemical properties of water in the breeding sites of mosquito larvae, this manuscript is composed of two parts main, the first is the bibliographic part of three chapters focused on general information on mosquitoes, larval breeding sites and their properties and finally the microbiological parameters of larval breeding sites; while the second part is the experimental part with two chapters on materials and methods and the second is results and discussion and finnaly with conclusion.

Chapter I General informations about mosquitoes

Chapter I

I. General informations about mosquitoes

Culicidae are a Diptera; one of the largest and most diverse orders of insects. The most significant group of biting Diptera comprises mosquitoes. This family is composed of arthropods belonging to the class of insects in the animal kingdom (Merabti, 2016) which are differentiated from other biting insects by their elongated, slender bodies, lengthy legs, and needle-like mouthparts.

Mosquitoes are present worldwide, and due to their remarkable adaptability and flight capabilities, they have been able to establish a global presence in areas with unfrozen water, which is crucial for their development (**Duvallet**, **2017**).

Currently, there are over 3,500 species of mosquitoes (Marquardt, 2004) which approximately three-quarters originate from subtropical and humid tropical zones (Boyer, 2017), Within the same genus, it is important to find local adaptations to prevent competition between species.

These insects pose a significant challenge to human health and socio-economic wellbeing. They can cause a variety of health problems due to their ability to transfer (vector) viruses and other disease-causing pathogens, even in the arid Southwest

1. mosquito morphology

The order Diptera comprises insects that have a single pair of membranous wings attached to the mesothorax, well-developed flight muscles in the mesothorax, a significantly reduced prothorax and metathorax, the latter of which carries the halteres, and a mouth part that can vary in size from 0.5 mm to 8 cm.

1.1 Egg

The eggs have a fusiform shape and measure between 0.5 and 1mm. Initially, they appear whitish in color but quickly turn black due to the oxidation of certain chemical components of the theca (**Peterson**, **1980**).



Figure 1. A mosquito egg (Loudet and al.2011)

1.2 Larvae

The larvae exhibit different cephalic characteristics, ranging from eucephalic to hemicephalic or vermiform (**Duvallet**, 2017). Their size varies from 2mm to 12mm, and they undergo four molts before becoming nymphs. The larval stage typically lasts for about 10 days and its duration is influenced by factors such as temperature, food resources, and the environment (**Boyer**, 2017).



Figure 2 a mosquito larvea(Hancock et al., 2022)

1.3 Nymph

Chapter I General information about mosquitoes

Or "the pupae" is a comma-shaped mobile stage showing a highly swollen cephalothorax and two respiratory trumpets. Notably, this stage is aquatic, short-lived, lasting 1 -5 days, and does not feed (**Peterson, 1980**). Furthermore, the nymphs are free and only occasionally enclosed in the pupal chamber.



Figure 3.morphology of Aedes aegypti Pupae (Rao et al., 2011)

1.4 Adult

or the imago which lives from one to twelve weeks, evolves in the aerial environment (**Delaunay** *et al.*, 2001). A tear opens the dorsal side of the nymph and the adult slowly emerges, it is made up of three parts: the head, thorax and abdomen wich are well differentiated and only have a pair of wings (Sérandour, 2007).

Males are recognizable by their feathery antennae, as opposed to females having glabrous antennae. Their first meal is composed of plant nectar (Sérandour, 2007; Boyer, 2017).



Figure 4 .a mosquito adult (Phan et al., 2020)

2. Classification

Mosquitoes are globally undesirable arthropods (El Ela *et al.*, 2024), Mosquitoes belong to the order Diptera, suborder Nematocera (Messai *et al.*, 2016), in the class of insects, suborder Nematocera and finally belong to the Culicidae family. This family currently includes around 3,500 different species (Duvallet, 2017).

Kingdom	Animalia
Sub kingdom	Bilateria
Phylum	Arthropoda
Class	Insecta
Order	Dipteria
Family	Culicidae
Genus	Aedes
Species	Aegypti

Figure 5. Systematic position of Aedes Aegypti (Muktar et al., 2016)

3. Life cycle

The mosquito life cycle is characterized by aquatic and terrestrial phases, and its duration depends mainly on temperature, microorganisms, water salinity and turbidity, light

exposure time, and mineral salts) (Mohammadkhani *et al.*, 2016), Their development goes through an aquatic larval phase before the aerial adult stage is interspersed with a short nymphal phase (Poupardin, 2011).



Figure 6. Life cycle of mosquito (Li et al., 2013)

4. Medicà veterinary importance

Of the 3,500 known species, only a tiny minority have adapted to human living conditions and colonized our towns and villages, or our places of recreation. They bite humans during the day or at night and are often responsible for major nuisances . if they have initially become infected by an infected host, Mosquitoes are thus involved in the transmission of dozens of human or veterinary diseases, some of wiche are infamous such as malaria and dengue fever (Sérandour, 2007). It is estimated that 725,000 people are killed each year by mosquito-borne pathogens .from around 300 different species of mosquitoes can bite humans around the world (Duvallet and Chabasse, 2020)

The most notorious is malaria (which kills 250 million people infected annually), which mostly children, mostly in tropical countries in Africa and Asia. Dengue fever kills over 30,000 people annually, mostly in Africa, and yellow fever kills about 30,000 people each year, mostly in Africa (**Boyer, 2017**).

Anopheles also play an important role in the health animals as potential vectors of *Plasmodiummammals* and birds, *Filariae* and viruses (*myxomatosis, Rift Valley fever*) (Duvallet and Chabasse, 2020).

Chapter II Physico-chemicals Properties of water of breeding site

5. Physicochemical Properties of water of the breeding site of mosquitoes

Stands as a crucial natural resource, playing a vital role in sustaining life for living organisms . It boasts numerous physicochemical properties, such as:

✓ Water temperature

Water temperature is an ecological factor that causes significant ecological repercussions (Leynaud, 1968). It affects the density, viscosity, and solubility of gases in water; the dissociation of dissolved salts; chemical and biochemical reactions; and the development and growth of organisms living in water, particularly microorganisms (Makhoukh *et al.*, 2011)

✓ pH (Hydrogen potential)

pH is a measure of the acidity of water. It measures the concentration of H⁺ protons contained in water (Makhoukh *et al.*, 2011). The pH scale extends in practice from 0 (very acidic) to 14 (very alkaline) (De Villers *et al.*, 2005) the pH of natural waters is generally between 6.6 and 7.8. However, in regions with acidic substrates or in water logged peatland areas, pH levels can be lower than 5. Conversely, in canals, slow-moving rivers, and certain ponds, the pH can momentarily reach values of 9 and even 10, depending on the significance of the photosynthetic activity of aquatic plants (Nisbet and Verneaux, 1970) Low pH (acidic water) notably increases the risk of the presence of metals in a more toxic ionic form, while high pH increases ammonia concentration (De Villers *et al.*, 2005)

✓ The electrical conductivity

Electrical conductivity (EC) is a numerical expression of the ability of a solution to conduct electric current (**De Villers** *et al.*, 2005) proportional to the quantity of dissolved ionizable salts; it constitue a good indication of the degree of mineralization of water (**Nisbet and Verneaux**, 1970), where each ion acts through its concentration and its specific conductivity (**Makhoukh** *et al.*, 2011). The electrical conductivity is expressed in amhos/an/ cm2 (accuracy1%) (**Nisbet and Verneaux**, 1970) or it expressed in millisiemensoer meter (ms/m) at 20° (**De Villers** *et al.*, 2005)

✓ Turbidity

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Cloudiness is a condition caused by the presence of suspended solids in water, such as soil particles, sand, clay, suspended organic and inorganic materials. It could also be due to the presence of bacterial microorganisms, floating plants, and soil colloids (El mousily, 2016)

✓ Dissolved oxygen

Oxygen is one of the particularly useful parameters for water and is an excellent indicator of its quality. It is one of the parameters most sensitive to pollution (Makhoukh *et al.*, 2011). The concentration of dissolved oxygen varies daily and seasonally because it depends on many factors such as the partial pressure of oxygen in the atmosphere, water temperature, salinity, light penetration, water agitation, and nutrient availability.Overall, the closer the dissolved oxygen (DO) concentration is to saturation, the greater the river's ability to absorb pollution:

- A value below 1mg of O₂ per liter indicates a state close to anaerobic.

- A value of 1 to 2 mg of O₂per liter indicates a heavily but reversibly polluted river.
- A content of 4 to 6 mg of O₂ per liter characterizes good quality water.

-Levels higher than natural oxygen saturation level indicate eutrophication of the environment resulting in intense photosynthetic activity (IBGE, 2002).

Chimical oxygen demand

Chemical oxygen demand represents the amount of oxygen consumed by chemically oxidizable materials contained in water. It includes the majority of organic compounds but also oxidizable mineral salts (sulfides, chlorides...) (Makhoukh *et al.*, 2011) and corresponds to the quantity of oxygen necessary for the chemical degradation, carried out using a powerful oxidant of organic organic compounds present in water.

Therefore, it is an important parameter for characterizing the overall pollution of water by organic compounds. (De Villers *et al.*, 2005)

Biochimical oxygen demand

BOD is the quantity of oxygen consumed by microorganisms in the dark at 20°C for 5 days. It allows the evaluation of biodegradable organic materials (Makhoukh *et al.*, 2011).In unpolluted waterways ,the BOD is very generally less than 3 mg/1 (Nisbet and Verneaux, 1970)

✓ Alkalinity expressed in mg/l of H CO-3

The variation in alkalinity should, therefore, be compared to that of the degree of mineralization (conductivity, alkaline earth content) and the carbon dioxide content (pH) for both the interpretation classes and the locations. However, total alkalinity also gives an indication of the degree of oxidation of organic matter (Nisbet and Verneaux, 1970)

✓ Chlorides

Chlorides are important inorganic anions contained in varying concentrations in natural waters, generally in the form of sodium chloride (NaCl) and potassium chloride (KCl) salts. They are often used as an index of pollution(**Makhoukh** *et al.*, **2011**). The chloride content of running waters, examples of pollution, hardly exceeds 20 mg/l (**Nisbet and Verneaux**, **1970**)

✓ Sulfate content expressed in mg/l of S04

This indication is especially useful for characterizing particular waters. The regional geological nature plays a very important role, as certain industrial and urban effluents can also provide water laden with sulfate (Nisbet and Verneaux, 1970).

Sulfate (SO4), one of the most common forms of sulfur in natural water, is the sulfate ion combined with forms of sulfur positive ions. It is included among the materials that contribute to salinity, which gives a salty taste when its concentration is more than 200 mg/l (El mousily, 2016).

✓ Nitrogen compounds

Nitrogen is an essential element for the construction of living cells. The forms of the nitrogen studied are ammoniacal nitrogen (NH4), nitrates (NO3), and nitrate (NO-3) (Makhoukh *et al.*, 2011)Ammonia nitrogen: is a water-soluble gas. There is a small proportion, less than 0.1 mg/L, of ammoniacal nitrogen in natural waters (Makhoukh *et al.*, 2011).

- 1. Nitrates (NO₃): are the final stage of nitrogen oxidation and are the most oxidized form of nitrogen found in water (**Makhoukh** *et al.*, **2011**) In impolluted natural waters, the nitrate level varies greatly depending on the season and the origin of the water; it can range from 1 to 15 mg/l, and a concentration of 2 or 3 mg/l is considered normal.
- Ammonia nitrogen: is a water-soluble gas. There is a small proportion, less than 0.1 mg/L, of ammoniacal nitrogen in natural waters (Makhoukh *et al.*, 2011).

✓ Calcium

In running waters, this content varies from 1 to 150 mg/l, mainly depending on the nature of the terrain that is crossed (Nisbet and Verneaux, 1970)

✓ Suspended or matter

It represents all the minerals and organic particles contained in water. They depend on the nature of the terrain covered, the season, the rainfall, the water flow regime, the nature of the discharges, (Makhoukh *et al.*, 2011)High levels of suspended solids can be considered a form of pollution (Makhoukh *et al.*, 2011).It includes all mineral or organic matter that does not dissolve in water, such as clays, sands, small organic and mineral particles, plankton, and other water microorganisms. The quantity of suspended matter varies, particularly according to the seasons and the water flow regime (De Villers *et al.*, 2005)

✓ Phosphate content expressed mg/l PO4

Phosphates are found in natural water and in domestic and industrial wastewater in dissolved or suspended forms (El mousily, 2016). The presence of phosphates in natural waters at concentrations greater than 0.1 or 0.2 mg/l is an indication of pollution by sewage water containing organic phosphate and synthetic detergents (Nisbet and Verneaux, 1970)

✓ Total hardness (TH)

The hardness is the ability of water to deposit soap. Soap in uncle s toilet due to the presence of divalent calcium and magnesium ions and metal ions like iron. Aluminum and zinic, as well as hydrogen, are either Carbonat, Bicarbonat, Clomid, and sulfat form (El mousily, 2016).

Chapter **I**

Chapter Ⅲ Microbiological charachteristic

6. Microbiological characteristics

The aim of identifying microbial content that could be useful for identification of potential larval breeding sites and ecologically inform mosquito control methods. Microbiological parameters (*total coliforms* (TC), *fecal coliforms* (FC) and *intestinal enterococci* (IE)) were enumerated by the membrane filtration method and the *total aerobic mesophilic flora* (FMAT) (Lalami *et al.*, 2010).

There is a certain specificity ensuring that there is a decoupling between the composition of the microbiome of the larva and that of the water in its habitat (Normandeau-Guimond, 2022); Bacterial content assessment of the breeding ponds revealed that the most abundant bacterial phyla were *Patescibacteria*, *Cyanobacteria*, and *Proteobacteria*, constituting >70% of the total bacterial richness. Microbes that are obtained during larval stages affect many physiological processes in the adult mosquito including susceptibility to various pathogens.

There are certain bacteria that had a toxic effect on insects. Toxins from two species of bacilli are used today against mosquito larvae: *Bacillus thuringiensis israelensis (BTI)* very effective, is quickly inactivated in polluted waters, and *Bacillus sphaericus* resists better in water loaded with organic matter (Guillaumot, 2005).

Ш

Experimental part

Chapter I Materials and methods

1. Study sites

Samples are collected from various potential mosquito breeding sites to study and identify the characteristics of mosquito larvae. This is carried out at specific times of the year depending on the species of mosquito and its reproductive cycle, using a variety of techniques employed in research and larval identification.

Mosquito larvae were gathered from stagnant oviposition sites, which are places with standing water throughout the sampling period. These sites included artificial pools, riverbank creeks, marshes, large metal containers, abandoned wells, water canals, and pits with plastic lining (Hassanin *et al.*, 2017)animal hoof prints (Emidi *et al.*, 2017)gutters, puddles, as well as natural containers like bamboo stumps, coconut shells, tree holes, and leaf axils. Additionally, mosquito larvae were collected from artificial containers such as clay pots, plastic cups, glass bottles, and tires (Gopalakrishnan *et al.*, 2013).

- The study carred by (El Ela *et al.*, 2024).was carried out in, Forty-two breeding sites, including canals, sewage tanks, agricultural puddles, stagnant water puddles, and swamps
- The study of (Ammar *et al.*, 2013)was carried out in two localities representing different levels of urban planning in Five types of the potential breeding habitats prevailing in the two localities (springs, cesspits, cesspools, irrigation ditches and drainagecanals
- The study of (Bouabida *et al.*, 2012)covers five of they, belonging to the same bioclimatic stage, the semi-arid, It focused on a sample of 16 deposits cistern, well, basin,marsh, valley, ditches
- In three sampling stations of (Messai *et al.*, 2016)(1, 2 and 3). The choice of these sampling stations is based on their position in relation to urban areas and especially for accessibility station 1: it is a pond with grassy vegetation with little algae and Station 2: it's a grassy marsh littered with seaweed, airy, sunny and full of deduction Station 3: it is located close to the previous breeding site
- Six breeding sites were identifiedby (Merabti and Ouakid, 2011) at each station The breeding sites were classified using the following criteria: nature (temporary or permanent),type (waste-water canal, tire, dam water, valley1grassy puddle,

discharge, irrigation basin, drainage ditch, marsh, lagoondepression, traditional basin water, faucet water, well water

- In the study of (Dahchar *et al.*, 2017)Three sites, each part of the site two stations
 The rural site is represented by 3 stables and two stagnations
- in the study area of (Oussad et al., 2021)was seven permanent sites, which differ ecologically from one another, Site 01: is an open water ditch, fed by spring and rainwater., The water of this ditch is rather clear and deep site 02 is a shallow cemented basin without vegetation when site 03: is rather a shallow shady channel with clear water and site 04: represents two drinking troughs located in Tassaft , The water in this breeding site is rather clear; while the 5th site is a water reservoir, which receives rainwater and spring water. Located at the entrance of Yatafen. The water in this gîte is turbid, shallow and polluted by domestic waste wherease Site 06: is a relatively large, shallow swamp in the Irdjen area, the water is rather clear and the last one is the site 07: is a shallow water channel fed by dam waters coming from the Irdjen area and rainwater. The water in this sunny breeding site is clear and covered with filamentous algae.
- As (Lalami *et al.*, 2010)Station I: Boufekrane Dam also called Gaâda,Station II: Green Diamond Bridge is an overflow ,Station III: Oued Fez ,Station IV: a small body of water of 1000 m2 fitted out with a fountain spread over 4 localities, which makes it a potential risk site. Station V: an intermittent deposit of 30,000 m2 linked to the irrigation waters of the Golf Royal, Station VI: Road to Sidi Harazem 5,000 m2 lodge, resulting from overflows from the region's irrigation seguias. this is the route taken by illegal African immigrants, potential carriers of the parasite and Station VII: as swimming pool.
- The study sites chosed by (Elmalih *et al.*, 2018) was three different sites of El Fetyhab area south Omdurman province, Khartoum State in Sudan they are Omdurman Islamic University area, Army Place and White Nile River Bank.

2. Techniques of sampling

• Larvae were collected by dipping method using an appropriate larval net made of a 20 cm diameter iron hoop connected to a 30 cm long muslin cover. Several different depressions (according to the width and depth of the breeding site) were carefully sampled from the surface. Larvae were collected from each site and transported alive to the laboratory in 500 ml plastic cups filled with breeding site water (Dahchar et al., 2017; Oussad et al., 2021; El Ela et al., 2024)

- The specimens were put into vials filled with 70% ethyl alcohol (Lalami et al., 2010)
- The dipper was dipped 10 times into each water sample at a construction site, with three minutes intervals between each dip (Deme *et al.*, 2017).



Figure 7. Dipping method to collect mosquito larvae in the study sites (Merabti, 2016)

3. Larval identification

There are different techniques used for the identification of larvae among which:

✓ Collected larvae are stored in labeled vials, Identificated and organizated by ethanol-containing deposits (70%), then marinated in KOH (10%) for 12 to 24 Hours of inspiration. This step is followed by rinsing. Then use distilled water (03 times, 2 to 5 minutes each). By increasing the alcohol concentration (70%, 90%, and 100%) for 15 minutes each concentration, First remove the moisture contained in the sample and then remove the larvae, to Place in beech creosote for at least 1 hour and assemble Balsam in Canada (Messai *et al.*, 2016).

✓ Each dip or site (N = 500) contained one hundred live third-instar larvae, which were used to identify the type of mosquitoes collected. A compound light microscope, Optic 10 lab (Ray Wild Limited Company, Germany), was used to observe the larvae. The morphological traits included in Harbach's graphic key were used to identify larvae (Elhawary *et al.*, 2020; El Ela *et al.*, 2024).

✓ Using identification software, the systematics of the Culicidae in the Tébessa region was primarily studied: de Bruhnes et al. (1999) for mosquitoes from Mediterranean Africa, and a dichotomous key from (Aouinty *et al.*, 2006) for mosquitoes existing in Morocco (Lalami *et al.*, 2010; Merabti and Ouakid, 2011; Bouabida *et al.*, 2012; Messai *et al.*, 2016).

4. Physicochemical analysis

Table 1 devices used in physicochemical analysis

Parameter	Material
ph	HANNA HI 8314 portable device/pH meter/ standard buffersolutions/pH probe (Eutech –cyberscan PH 5500) (augste 2017)
T°	HANNA HI 8314 portable device/Thermometer or thermometric methode or mercury thermometer
	standard methods of American Public
DO	Health Association/Polarigraphique (type W.T.W.oxy 39) /dissolved oxygen kit / dissolved oxygen meter
Turbidity	turbidimeter HI 98703/Ultraviolet Spectrophotometer Screenig Method or using Standar solution (APHA 1985)
	conductivity/TDS
TDS: total dissolved solid	meter (HACH, HQ440d multi) (HACH Company, Europe)/Turbidimeter device (2100P Portable Turbidimeter at Hach) (Augste 2017) or Nephelometric Method (APHA 1985) or saki disc
Alkalinity	titration technique
CL⁻: chlorure	titration technique/Mohr or Clarke method (1950) change by Domask and Kok (1952)

Chapter I Materials and method

EC: electrical conductivity	conductivity/TDS meter (HACH, HQ440d multi) (HACH Company, Europe)/Conductometer device (Elytic – Aqua con 200) (Augste 2017)
NO ² : Nitrite	spectrophotometer standard environmental protection agency methods/Ultraviolet Spectrophotometer Screenig Method or using Standar solution (APHA 1985)
phosphate	Deniges (1930) or spectrophotometer device (Perkinelmer UV/Visible lambad EZ 201 and Hach) (Augste 2017) or Asorbic Acid Method (Tandon 1998)
BOD	Aqualtic (APHA 1985)
Viscosity	Viscometer
Metieres en suspension	Filtration then thought of the residue dried in the oven at 105 °C and cooled (accuracy 2%)

We can using Orion 5-star portable multiparameter (Thermo Scientific) for measured PH-conductivity-salinity – total dissolved solids – tubidity and dissolved oxygen or meter (HANNA Instruments ,United States)

5. Statistical analysis

Data were processed for statistical analysis using SPSS (version 26) and Microsoft Excel worksheets (Microsoft Office 2010) for Windows (El Ela *et al.*, 2024)

The differences in the physicochemical parameters and species percentage among sites were tested using Kruskal-Wallis test (Elhawary *et al.*, 2020).

The frequencies of occurrence for each mosquito species reported were compared to the characteristics of their breeding habitats through calculation and analysis. Contingency tables of 2x2 and 2x3 were created, and $\chi 2$ tests were conducted to assess the relationship between species occurrence and specific characteristics. A significance level of 5% was

applied, and statistical analysis was performed using the SSP (Smiths Statistical Package) computerized software developed by Smith in 2004 (Ammar *et al.*, 2013).

We employed one- and two-factor analysis of variance (ANOVA) along with principal component analysis (PCA) for the statistical study. Upon rejection of H0 latter, To establish homogeneous groups, the Fisher LSD test is employed ;The primary goal of the PCA is to visually depict the majority of the data in a range of quantitative data. Individuals in a table with j data variables are in a d-dimensional space.

Finding smaller spaces that minimize these deformations is the task of PCA (Messai *et al.*, 2016).

Chapter I Results and discussion

1. Physicochemicals analysis of water breeding sites

After studying the reproduction of mosquito larvae inseveral regions of the world, we found several types thenumber of which varies according to the location of eachregion and according to the rainy season or the dry season aswell as according to the effect of the physicochemical factors of the water.

The following results in the papers analysed were as follows:

✓ Temperature

Through the introduction studies on the reproduction of mosquito larvae we find that temperature has a significant impact on this; T° from 19 °C to 29.8 °C (El Ela et al., 2024) find that Oc. caspius and Cx. pipiens were the only two species recorded in the breeding sites and with 32.00 ± 7.42 a in Al-Beshlawy Drainage canal (Elhawary *et al.*, 2020) recorded A total of 1500 larvae were sampled Five Culicine species were identified: Culex pipiens Linnaeus (95.0%), Cx. univittatus Theobald, Cx. antennatus (Becker), Cx. quinqueifasciatus and Cx. perexiguus when in Tanta manmade ground hole and ElKhartoum irrigation ditch ($T^{\circ}:85.6 \pm 4.2b$ and 28.63 ± 5.83 respectively) Five species were identified They were Cx. Pipiens, Cs. longiareolata Macquart, Cx. univittatus, Cx. Antennatus, and Cx. quinqueifasciatus, results of (Messai et al., 2016) showed the presence of seven species belonging to two subfamilies: Anophélinae and Culicinae. The T° recordes in the three stations is $26.00 \pm 5.83 \ 23.33 \pm 8.06 \ 24.78 \pm 7.48$ respectively with presence of Aedes dorsalis, Aedes detritus, Aedes caspius, Aedes mariae, Aedes vexans, Anopheles sergenti, Culiseta longiareolata where (Oussad et al., 2021) obtained the following results, the average water temperature recorded is 10.06 °C and 28.32 °C : Culiseta longiareolata, Culex pipiens, Culex hortensis, Culex impudicus, Anopheles labranchiae 0.42± 0,00, Culex perexiguus and Anopheles claviger in other side (Lalami et al., 2010) find that The majority of the breeding sites under study have water temperatures above 20.4°C, which is ideal for anopheles larvae to develop. In June, it reaches its highest point (31.4°C). At stations II, VI, and VII, the difference between the maximum and minimum is 3.6 to 4.6 °C, at stations V and III, it is 7 °C, and at station I, it is 9.2 °C. There are slight temperature fluctuations (difference of 1.2°C) at station IV; in T°=24-27 A. gambiae complex, A. pharoensis, C. quinquefasciatus, C. univittatus, C. Theiler and C. pipiens this results is recorded by (Elmalih et al., 2018); Where we found the temperature is from 10°C to 18°C depending

(Hassanin et al., 2017) the An.claviger An.hyrcanus An.maculipennis s.] An.pseudopictus Cx.torrentiumCx.hortensis Cx.annulata but when the temperature is between 23.1°C to 27.2 ° C (Soleimani-Ahmadi et al., 2014) it is related to the following larval density like An.culicifacies, An.dthali, An.superpictus An.stephensi, An.moghulensis, An.turkhudi and the espies Ae.albopictus (Rao et al., 2011). There was no significant negative correlation An.marteri, the temperate and larval abundance like between *Cx.pipiens*. Cx.tritaeniorhynchus, Cx.territans Cx.mimeticus, Cx.perexiguus, Cx.longiareolata and Cx.morsitans (Hassanin et al., 2017)

✓ pH

In addition, pH is negatively correlated with presence of mosquito's larvae that is what find (Abdalla et al., 2022), but (Lalami et al., 2010) The pH of the water in the lodges is alkaline due to the calcareous nature of the terrain crossed just Anopheles labranchiae was found, (Messai et al., 2016) also found a negative correlation between pH and presence of mosquito's larvae collected, when (El Ela et al., 2024) found that Oc. caspius and Cx. *pipiens* were the only two species recorded in the breeding sites at pH 5.5 (acidic medium) while data revealed a negative correlation between and Cx. Antennatus and pH, Cx. Perexiguus was positively associated with pH; the pH (6.72±0.11 to 7.63±0.18) showed positive correlation with the larval density Ae.aegypti ,Ae.albopictus, Cx.quinquefasciatus, Ar. subalbatus, Toxortynchites, Lutzai spp. (Gopalakrishnan et al., 2013); -in the same field of PH, we find other spieses of larval likeAn.claviger An.hyrcanus An.maculipennis s.l An.pseudopictus Cx.torrentium Cx.hortensis An.marteri, Cx.morsitans (Hassanin et al., 2017) and results concerning the chemical characteristics of the larval habitas showed that larvae of allanopheline species (An.culicifacies, An.dthali, An.superpictus An.stephensi, An.moghulensis, An.turkhudi had beencollected from water bodies with mean pH ranging from 7.1 to 8.6 (moussa, hassane and al. 2014) In other studies, it has been shown that the Ph has a negativecorrelation with larval density : Ae.albopectus (B Bhaskar , PsHarikumar and al.2011), An.gambiae An.funestus and Culex spp. (Lawal et al., 2022) and Cx.pipiens, Cx.tritaeniorhynchus, Cx.territans Cx.mimeticus, Cx.perexiguus, Cx.longiareolata and Cx.annulata (Hassanin et al., 2017)

✓ Turbidity

Results found by (Elhawary, Soliman et al. 2020) showed that *Cx. Perexiguus* was positively associated with it, turbidity may partially be due to the presence of solid wastes (Ammar *et al.*, 2013)*Cx. perexiguus* and *Cx. pusillus* prefer breeding (P<0.05) in water with

solid wastes, whereas Culex quinquefasciatus was highly Significantly correlated with water turbidity, $(r^2=0.29832, n=168, P \le 0.001)$ and C. theileri was significantly negatively correlated (r2=-0.18737, n=168, P≤0.05) Anopheles gambiae complex was reared with the highest mean in water turbidity of (451-550 ppm) and the lowest in (351-450 ppm) (Elmalih et al., 2018); -Turbidity was found to be significant (p<0.05) and these can associated to the larval abundance and density, where if the turbidity ranging from $(7.8\pm0.2 \text{ to } 28.1\pm0.7)$ NTU) we found An.culicifacies, An.dthali, An.superpictus An.stephensi, An.moghulensis, An.turkhudi (moussa,hassane and al.2014) and the espies Ae.albopictus (Rao et al., 2011) And the turbidity ranging from (12.50±0.70 to 97.00 NTU) we found An.claviger. An.hyrcanuş An.maculipennis s.l. Cx.torrentium An.marteri, Cx.morsitans Cx.pipiens, Cx.territans Cx.mimeticus, Cx.perexiguus, and Cx.annulata (Hassanin et al., 2017). it ranging from 28.0 to 215.0 NTU showed positive correlation with larval density Ae.albopictus (Rao et al., 2011) but it showed negative correlation with larval density like Ae.aegypti , Ae.albopictus, Cx.quinquefasciatus, Ar.subalbatus, Toxortynchites, Lutzai spp.(Gopalakrishnan et al., 2013) and Cx.tritaeniorhynchus An.pseudopictus Cx.hortensis and Cx.longiareolata (Nikookar et al., 2017)

✓ Electrical conductivity

Between 595,3and 1549,6 (µs/cm) is important for presence of Anopheles labranchiae, Culex hortensis, according to (Lalami et al., 2010) also the productivity of certain species such as Culex pipiens, Culiseta longiareolata, Culex hortensis and Anopheles labranchiae seems to be positively correlated to conductivity, whereas the productivity of Culex perexiguus, Culex impudicus and Anopheles claviger is negatively and insignificantly correlated to conductivity. Knowing that Average conductivity ranged from 0.2627 at deposit 02 to 1.19 mS/cm at deposit 03.(Oussad et al., 2021); An.claviger, Cx.torrentium An.marteri, Cx.morsitans Cx.pipiens, Cx.mimeticus, and Cx.annulata, Cx.tritaeniorhynchus and Cx.hortensis were found in breeding sites with conductivity ranging from 419 to 1430.86±1395 Us/cm (Hassanin et al., 2017)An.gambiae, Culex spp had been collected from water bodies with conductivity ranging (81.2 ± 4.00 to 151.2 ± 4.60) and (101.3 ± 13.33 to 203.1±11.30)respectively but An.funsetus showed negative correlation with conductivity (Lawal et al., 2022) and we found An.culicifacies, An.dthali, An.superpictus An.stephensi, An.moghulensis, An.turkhudi with conductivity ranging 941 to 2424.6 uS/cm (Soleimani-Ahmadi et al., 2014) .Ae.albopictus collected from water bodies with conductivity 73.6 to 1.415.9 Us/cm (Rao et al., 2011).water conductivity with a range of 162.9 to 616.9 uS/cm

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, was observed to have significant negative correlation with the larval density Ae.aegypti , Ae.albopictus, Cx.quinquefasciatus, Ar.subalbatus, Toxortynchites, Lutzai spp.(Gopalakrishnan et al., 2013)

✓ Dissolved oxygen

DO (Dissolved oxygen) *Ae.aegypti* ,*Ae.albopictus*, *Cx.quinquefasciatus*, *Ar.subalbatus*, *Toxortynchites*, *Lutzai spp*.were found in breeding sites with DO ranging 1.11 ± 0.06 to 2.11 ± 0.72 % (Gopalakrishnan *et al.*, 2013)and we found*AN.gambiae* and *An.funestus* in DO ranging(9.3 ± 2.00 to 16.0 ± 4.80 mg/l) and 25.2 ± 6.00 respectively but *Culex spp*. (Lawal,Idoko and al.2022) ; when (Oussad *et al.*, 2021) shows that larval productivity is negatively correlated in an insignificant way with it knowing that DO recorded at the study deposits is between 3.577 and 8.82 mg/L

✓ Nitrite and nitrate

(El Ela et al., 2024) and with (0.13-5.26) found that The larvae of An. sergentii showed the highest intolerance to nitrite concentrations Cx. pipiens, Oc. caspius, and Cs. longiareolata larvae favor habitats with low levels of nitrite (about 0.6 mg/l); however, Cx. perexiguus and Cx. pusillus tolerate higher levels (up to 25 mg/l) when data revealed a negative correlation between this species and and NO2 plus whereas Cx. quinqueifasciatus, it was inversely related to nitrite but Cs.longiareolata had a positive association with it (0.20-0.98) according to (Elhawary et al., 2020) whereas Aedes dorsalis is positively correlated to it (r = 0.507) when *Aedes* detritus is negatively correlated to nitrite (Messai *et al.*, 2016); The concentration of nitrate was variable among different larval habitats ranging from 0.30 to 5.23 mg/l and was positively correlated with larval density An.marteri, *Cx.pipiens*, *Cx.mimeticus*, *Cx.annulata*, *Cx.tritaeniorhynchus*, Cx.perexiguusCx.hortensis,An.pseudopictus,Cx.perexiguis,An.hyrcanus and An.maculipennis

s.1 (Hassanin *et al.*, 2017). And with larval density *Ae.albopectus* with ranging 1.1 to 4.7 mg/l (Bbhaskar, PS Harikumar and al.2011) and *An.gambiea* was found in breeding sites with concentration of nitrat ranging from 15.0±1.22 mg/l (Lawal *et al.*, 2022)

✓ Chloride (Cl⁻)

At (80-1340)mg/l It is worth mentioning that there was a significant positive correlation between the prevalence of both *Oc. caspius and Cx. antennatus* and Cl⁻ In the same regard, Cx. pipiens showed negative correlations with it plus f *Cx. Perexiguus* according to (El Ela *et al.*, 2024); when (Messai *et al.*, 2016) showed that The larval density of *Aedes dorsalis* is negatively correlated with Cl⁻ (r = -0.415) when *An.claviger*.

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An.marteri, Cx.morsitans Cx.pipiens, Cx.mimeticus, Cx.annulata , Cx.tritaeniorhynchus , Cx.hortensis , An.pseudopictus , An.hyrcanus Cx.perexiguus and An.maculipennis s.1 were found in breeding sites withchloride ranging from 45.98 to 826.24 ± 1751.24 mg/l (Hassanin *et al.*, 2017).concentration of chloride between 21.1 ± 5.57 to 2.6 ± 0.05 and 14.2 ± 3.3 mg/l related to the larvaldensity of *Culex spp* and *An.gambiea* s.1 respectively (Lawal *et al.*, 2022).

✓ TDS: total dissolved solids

Oc,caspius showed the highest tolerance to the higher values of TDS as well *Oc. caspius* and *Cx. Antennatus* In the same regard, *Cx. pipiens* showed negative correlations with it (276–5344) (mg/l) (El Ela *et al.*, 2024) ; *Ae.albopectus* was found in breeding sites with TDS 52.4 to962.9 mg/l (Rao *et al.*, 2011) and TDSwas positively correlated with larval density *An.gambiea* withranging 23.4 \pm 5.13 mg/l (Lawal *et al.*, 2022). but theseparameter was not significantly associated with larval d*ensityAn.culicifacies*, *An.dthali*, *An.superpictus An.stephensi*, *An.moghulensis*, *An.turkhudi* (Soleimani-Ahmadi *et al.*, 2014) and *Culex spp* and *An.funestus* (Lawal *et al.*, 2022)

✓ Alkalinity

Ae.albopictus collected from water bodies with alkanity 82.4to 804.8 mg/l (Rao et al., 2011) and wefound An.culicifacies, An.dthali, An.superpictus An.stephensi, An.moghulensis, An.turkhudi with water alkanity ranging 142.6to 523.2 mg/l (Soleimani-Ahmadi et al., 2014) and An.claviger, Cx.torrentium An.marteri, Cx.morsitans Cx.pipiens, Cx.mimeticus, and Cx.annulata, Cx.tritaeniorhynchus, Cx.hortensis and Cx.longiareolata were found in breedingsites with alkanity ranging from168 to 500 mg/l (Hassanin et al., 2017).but the alkanity was not significantlyassociated with larval density : An.hyrcanus, An.maculipennis s.l ,An.pseudopictus, Cx territans, Cx.perexiguus and Cx.longiareolata (Hassanin et al., 2017)

✓ TH (Total hardness)

The concentration of TH was variabl among different larvalhabitas ranging from 33.5 to 563.6 mg/l and it was positivelycorrelated with larval density Ae.albopectus (Rao et al., 2011) and with larval density An.marteri, Cx.morsitans Cx.pipiens, Cx.mimeticus, Cx.annulata , Cx.tritaeniorhynchus Cx.perexiguus and Cx.hortensisAn.pseudopictus, Cx.perexiguis and Cx.annulata (Nikookar et al., 2017). ranging from 192 to 716 mg/ butthese parameter was not significantly associated with larval density

An.culicifacies, An.dthali, An.superpictus An.stephensi, An.moghulensis, An.turkhudi (Soleimani-Ahmadi et al., 2014)

✓ BOD (Biological oxygen demand) and COD (chemical oxygen demand)

Culex.spp, *An.gambiea* and *An.funetus* were found inbreeding sites with concentration of BOD ranging from 29.6 \pm 7.65 mg/l,48.3 \pm 8.22 mg/l and 50.4 \pm 4.00 mg/lrespectively (**Lawal** *et al.*, **2022**)*Culex.spp*, *An.gambiea* and *An.funetus* were found inbreeding sites with concentration of COD ranging from 3.3 \pm 0.17 to 6.5 \pm 0.3mg/l ,9.3 \pm 1.20 mg/l and 13.8 \pm 2.00 mg/lrespectively (**Lawal** *et al.*, **2022**)

✓ Phosphat

An.marteri, Cx.morsitans, Cx.mimeticus, Cx.annulata, Cx.perexiguus, Cx.hortensis. An.claviger, An.hyracanus, An.macuipnnis, An.pseudopictus, Cx. perexiguis and Cx.annulata. (Hassanin et al., 2017) were found in breeding sites with concentration of PO_4 -ranging from 0 to 3.2 mg/l and An.gambiea and Ae.albopectus were found with concentration of PO_4 -ranging from 2.5 to 0.03 mg/l and 0.02 to 10.3 mg/l respectively (Rao et al., 2011; Lawal et al., 2022)

✓ Calcuim

The concentration of calcium was variabl among different larval habitas ranging from 12.4 to 122.7 mg/l and it waspositively correlated with larval density *Ae.albopectus (Rao et al., 2011)* but these parameterwas not significantly associated with larval density *An.culicifacies, An.dthali, An.superpictus An.stephensi, An.moghulensis, An.turkhudi* (Soleimani-Ahmadi *et al., 2014*) and with *Culex spp , An.gambiae* and *An.funestus* (Lawal *et al., 2022*)

✓ Sulphat

The concentration of sulphat was variable among different larval habitats ranging from 2.9 to 85.6 mg/l and 14.18 to 446.18 mg/l and 166.5 to 485.8 mg/l it was positively correlated with larval density *Ae.albopictus* (Rao et al., 2011), *An.marteri, Cx.morsitans*, *Cx.pipiens, Cx.mimeticus, Cx.annulata*, *Cx.tritaeniorhynchus* Cx.perexiguus and Cx.hortensis (Hassanin et al., 2017)*An.culicifacies, An.dthali, An.superpictus An.stephensi, An.moghulensis, An.turkhudi* (Soleimani-Ahmadi et al., 2014) respectively but this parameter was not significantly associated with larval density *An.claviger, An.hyracanus, An.macuipnnis, An.pseudopictus, Cx. perexiguis* and *Cx.annulata* (Hassanin et al., 2017)

2. Microbiology analysis of water breeding sites

The microbiological study of the roosts revealed low to significant bacterial contamination of human and/or animal origin.

Total aerobic mesophilic flora TAMF and AEs having no effect on the proliferation of larvae; when record maximum values of total coliforms in breeding sites of *Anopheles labranchiae* and *Culex hortensis*, the latter record a minimum of Fecal coliforms also(Lalami *et al.*, 2010); study sites of (Abdalla *et al.*, 2022)showed the presence of three bacterial growths: Coli form, thermo tolerant coli form and E. coli.

3. Discussion

This study was conducted to investigate the correlation between mosquito relative abundance and their habitats' physicochemical parameters. From results showed we can conclude that *Culex* larvae are found more frequently in waters rich in organic matter and ammonium, with high temperatures and low conductivity (Lalami *et al.*, 2010) ;*Culex pipiens* is always correlated positively with T° and pH (depend on (El Ela *et al.*, 2024)these parameters appear to be important in the appearance and fluctuation of mosquito larval populations (Oussad *et al.*, 2021); alkalinity, TH conductivity and sulfat in the same time it showed a negative correlated with T° and water turbidity but it was inversely related to nitrite; *Cx. Antennatus* too have a positive correlation with T°, TDS and Cl⁻; *Culex perexiguus* is positively correlated with T°, pH ,Turbidity, but not conductivity or nitrite, when *Cx.hortensis* has a positive correlation just with T° (there is a lot of species which according to just one parameter)

The presence of Aedes genera (*Aedes dorsalis ,Aedes detritus ,Aedes caspius ,Aedes mariae ,Aedes vexans*) showed positive correlation with T° ,pH, turbidity , alkalinity and phosphate but a negative one with conductivity and nitrite

Anopheles is one of the most abundance subfamily represented in An. plumbeus, An. maculipennis, An. claviger and An. sergentii species (Anopheles pharoensis and An. sergentii are the proven malaria vectors) and that due to his tolerated with several parameters like T°, pH, conductivity ...whereas Anopheles larvae have higher oxygen requirements than Culex or Aedes larvae (Trari and Himmi, 1987; Piyaratne *et al.*, 2005) indicated that in

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turbid breeding sites, *Anopheles* larvae are much more likely to be absent Typical habitats for Anopheles larvae are partly sunlit, pools ranging in size from foot prints, to ponds, to slow moving streams (Kenea *et al.*, 2011)showed that Anopheles species were most abundant in habitats with vegetation (Graves *et al.*, 2011)showed that the presence of vegetation can help larvae to hide from their predators. Thus, such factors should be considered when designing an integrated vector control programme.

Bacteriological proprieties too have a principal effect on the breeding sites of mosquito larva's whereas the presence of each of *total* and *fecal coliforms* associeted to presence of some *Culex* and *anopheles* species in addition to *Coli form* and *E.coli* existing in the breeding site of some species.

Conclusion

Conslusion

In this context, research assessing how both of phicochemical and microbiological of water influence on breeding sites of mosquito.

At the end of study of this research we can conclude that T°, pH, conductivity and DO are the best indicator for abundance of major of genera like *Culex*, *Aedes* and *Anoheles* and this proprieties has a positive correlation with this species, in addition to other parameters like TDS,Cl⁻,TH and salinitythese properties also have an effect on the larvae but not all species just a few.

Indeed, *Cx. pipiens* and *C. perexigius* grow in all types of breeding sites.We discovered genera of bacteria (Coli form and E.coli and thermo tolerant coli form) in larval habitats that have also been found in the midguts of Anopheles adults and larvae. This resultimplies that the bacteria are taken up by larvae from the environment and passed on to the adult stage, where they may be used as symbionts for defense or nutrition in mosquitos.

These data would be of great interest in the context of developing a program to control nuisances and prevent vector-borne diseases; In order for targeted mosquito species, particularly those that serve as disease vectors, to be effectively controlled via environmental control strategies focused on altering habitat characteristics.

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Abstracts

الكلمات المفتاحية: الخصائص الفيزيوكميائية الميكروبيولوجية, يرقات بعوض, الكيولكس والأنوفيل والإيدي

Abstract: There are many types of insects in large numbers, and each type of insect has its own unique morphological characteristics. The growth and reproduction of insects are influenced by the physical, chemical, and microbiological characteristics of the environment in which they live. In this presented research, an analytical study was conducted on numerous researches and studies in this regard. The distribution of mosquito species was studied in accordance with the physical, chemical, and microbiological characteristics of water, where samples of mosquitoes were taken several times from various environments using the dipping method. Based on the obtained results, it was found that natural water bodies, water pools, containers, and river edges are the most common habitats for mosquito larvae, where larval density is associated with water temperature (T°), pH, turbidity, conductivity, chloride, and positive oxygen (chloride and DO). Thus, we were able to determine the distribution of many species of mosquitoes from the Culicidae, Anophelinae, and Aedinae families. Microbiological analysis of water samples from breeding sites revealed the presence of three bacterial species: Escherichia coli, total coliforms, and thermotolerant coliforms. In conclusion, all final results indicate a strong relationship between physical and chemical characteristics and larval density.

Keywords: Physicochemical and microbiological properties, mosquito larvae, Culicidae, Anophelinae, Aedinae.

Resumé : Les insectes ont une grande diversité dans la nature, chaque type d'insecte a ses propres caractéristiques morphologiques uniques. La croissance et la reproduction des insectes sont influencées par les caractéristiques physiques, chimiques et microbiologiques de l'environnement dans lequel ils vivent. Dans cette initiative de recherche, une étude analytique a été menée sur de nombreuses recherches et études à cet égard. La distribution des espèces de moustiques a été étudiée en fonction des caractéristiques physiques, chimiques et microbiologiques de l'eau, où des échantillons de moustiques ont été prélevés plusieurs fois dans divers environnements à l'aide de la méthode de l'immersion. Sur la base des résultats obtenus, il a été constaté que les plans d'eau naturels, les piscines, les conteneurs et les rives des rivières sont les habitats les plus courants pour les larves de moustiques, où la densité larvaire est associée à la température de l'eau (T°), au pH, à la turbidité, à la conductivité, au chlorure et à l'oxygène positif (chlorure et DO). Ainsi, nous avons pu déterminer la distribution de nombreuses espèces de moustiques des sites de reproduction a révélé la présence de trois espèces bactériennes : Escherichia coli, les coliformes totaux et les coliformes thermotolérants. En conclusion, tous les résultats finaux indiquent une forte relation entre les caractéristiques physiques et chimiques et la densité larvaire.

Mots-clés : Propriétés physico-chimiques et microbiologiques, larves de moustiques, Culicidae, Anophelinae, Aedinae.