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Sterile _insect methods for control of some mosquito species

Jury :

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We extend our gratitude to the jury for bestowing upon us the honor of evaluating and assessing this humble work, lending value to our efforts through their judicious evaluation.

Dedication

To the soul of that wonderful man who made me proud to be his daughter, to my role model in life, my dear father

To the beautiful woman who gave me everything beautiful and surrounded me with her prayers wherever I went, to my dear mother

To my support and source of security, to all my brothers and to their children, our family's sweets, Hajar, Abdel Rahman, Leila and Elias

To all my relatives and extended family,

To everyone who taught me a letter or gave me advice,

To my friends who shared my sorrows and joys

I share this happiness with you

Asma

To complete my joy, I would love to share it with my loved ones and those close to my heart:

To the pure soul of my father, the greatest love in my life, who is the main reason for what I am today and the cause of any future success in my life.

To my dear mother, who always strives to be the perfect mother for us, May God keep you as a crown on our heads, and may He protect and bless you from all harm.

To my siblings who were blessings of brotherhood and extremely exemplary, I am very proud of them.

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To all members of my family.

To all my friends for their love, respect, humility, and for giving me positive energy.

I am truly fortunate to have you all, and may God protect you all for me.

Rima

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List of abbreviations

Cx: Culex

Ae: *Aedes*

An: *Anopheles*

PgSIT: Precision guided sterile insect technique

SIT: sterile insect technique

γ: Gamma

L1: First larval instars

L2: Second larval instarss

L3: Third larval instars

L4: Fourth larval instars

LD: Lethal dose

SEM: Scanning electron microscope

Ix: *Intersex* gene

BTub : *β-Tubulin 85D*

Dsx : *doublesex*

Cas 9: CRISPR associated protein 9

CRISPR: Clustered regularly interspaced short palindromic repeats

Cx. pipiens : Culex pipiens.

Cx. p. quinquefasciatus : Culex pipiens quinquefasciatus.

An. stephensi: Anopheles stephensi.

Ppm: Partie par million.

WAlbB: w stands for Wolbachia and AlbB denotes the specific strain of the bacteria.

LR [wPip (Is)]: stands for LR line infected with Wolbachia wPip(Is.)

IIT : Incompatible Insect Technique.

Introduction

Introduction

Mosquitoes, members of the Culicidae family, are small flying insects **(Morales and Villanueva, 2020)**both male and female mosquitoes feed on nectar, but only females require a blood meal for egg production **(Guillaumot, 2006)**, they possess specialized mouthparts, called proboscis, to pierce the skin of their hosts and extract blood**(Morales and Villanueva, 2020)**, mosquito larvae develop in stagnant water, where they undergo several stages of growth before emerging as adults **(Adisso et Alia. 2005)**.

Mosquitoes, or Culicidae, are indeed of paramount importance in human and animal health, their main role lies in their ability to act as vectors of infectious agents, thus transmitting various diseases to humans and animals., among the most notable diseases transmitted by mosquitoes is malaria, caused by the parasite *Plasmodium*, this infectious disease is responsible for millions of deaths each year, making it a major public health problem in many regions of the world**(Nebbak et al., 2022)**, mosquito bites can also transmit other serious diseases such as dengue fever, Zika virus, chikungunya, and yellow fever, making mosquitoes a serious threat to global public health **(Guarner and Hale 2019) ,**Viral diseases, particularly arbovirus infections like dengue fever, are highly prevalent, dengue fever, undoubtedly one of the most common, sees 50 to 100 million cases annually, including hundreds of thousands of cases of hemorrhagic dengue, which is particularly deadly **(Gubler, 2002)**,it seems you're discussing various mosquito-borne diseases, including yellow fever (FJ), Rift Valley fever (FVR), West Nile virus fever (VWN), chikungunya, various viral encephalitis, and nematodes such as those causing filariasis., altogether, fewer than 150 species of mosquitoes belonging to the genera *Anopheles*, *Aedes*, and *Culex* are indirectly responsible for morbidity and mortality in humans, more than any other group of organisms **(Harbach, 2007)**, mosquitoes play a vital ecological role as they contribute to various ecosystems, aiding in nutrient cycling and serving as a significant food source for other organisms**(Ferraguti et al., 2021)**.

Sterilization methods are crucial for controlling mosquito populations and reducing the transmission of diseases like dengue and Zika, there are many sterilization methods**(Urquidi et al., 2015)**, including the bacterium *Wolbachia*, *Wolbachia*, a bacterium, is introduced into mosquitoes, interfering with their reproduction and leading to non-viable eggs**(Sakamoto and Rasgon, 2006)**, the Sterile Insect Technique (SIT) involves releasing sterile male mosquitoes, produced through irradiation or genetic modification, to mate with wild females, suppressing the population**(Dyck et al., 2021)**, chemosterilants disrupt mosquito reproductive processes**(KS, 1964)**, while precision-guided SIT utilizes genetic modification to create sterile males **(Gendron et al., 2023)**, these diverse approaches aim to effectively curb disease transmission and combat insecticide resistance, highlighting the importance of sterilization methods in integrated mosquito management strategies**(Urquidi et al., 2015)**.

The aim of this study is to determine the best technique for sterilizing the three selected species of mosquitoes among the four techniques we studied.

Our manuscript is composed of four parts, an introduction which covers the general context of the study and the problem, in second order, biological materials consist of mentioning some general and necessary characteristics and concepts about the Culicidae family at the end of this part, we chose three species of Culicidae, *Aedes aegypti*, *Culex pipiens*, and *Anopheles stephensi* in order to apply and test the effectiveness of the selected sterilization techniques on them, then, in the second chapter, we chose four methods for sterilizing mosquitoes and talked about them briefly and simply, mentioning the pros and cons of each technique, in the third chapter, we talked about the work methodology and chose 18 scientific articles studied the effectiveness of these four techniques on the three species of mosquitoes in different geographical areas and environments, the fourth chapter consisted of results and discussion .Finally, the conclusion in which we answered the problem that we raised in the introduction.

Bibliographic part

chapter IBIOLOGICAL MATERIEL

CHAPTER I BIOLOGICAL MATERIEL

1 History

At the end of the nineteenth century, much information had been known about mosquito biology, as adult feeding mechanisms were described. The stages of the life cycle internal anatomy and histology, egg-laying behavior, and the development of its classification. knowledge of the important role of mosquitoes in transmitting pathogens led in the last two decades to the launch of many campaigns to collect and classify it , where was Theobald able to define the genera through the arrangement and shape of the scales in adults , and both of Dyar and Knab distinguished subfamilies by the characteristics of the larvae **(Clements, 2012)**.

2 Definition

Mosquito are from Culicidae family and order Diptera **(Morales and Villanueva, 2020)** and there are more than 3546species **(Merabti, 2016)** that have been divided into 39 genus **(Crans, 2004)** its characterized by their slender body with long legs , the proboscis which is an elongated part of the mouth , is what facilitates the process of identifying them . they are also distinguished by scales , veins and wings , their importance is the ability of females to transmit diseases **(Morales and Villanueva, 2020)**.

3 Classification

King dom: Animalia

Sub-kingdom: Metaoa

Phylum: Arthpoda

Sub-phylum: Hexapoda

Class: Insecta

Sub-class: Pteregota

Order: Diptera

Sub-order: Nematocera

Family: Culicidae

Sub- families : Culicinae **(Boyer, 2006).**

4 Morphology General morphology of Culicidae

The Culicidae are holometabolous dipterans, meaning they undergo complete metamorphosis. Their development progresses through four stages: egg, larva, and pupa, which are aquatic stages, before reaching the adult aerial stage.

A) The egg

After mating, the female mosquito requires a blood meal to allow her first batch of eggs to mature. These eggs are surrounded by a thick shell with a micropyle at its anterior end. Traditionally, the egg of Culicidae consists, from the inside out, of the embryo, the pellucid vitelline membrane, the thick endochorion, and the more or less pigmented, pitted, or areolate exochorion (Rioux, 1958).

The number of eggs laid can reach 300 depending on the species, and they hatch within 24 to 48 hours **(Ripert 2007)**

B) The larva

Mosquito larvae require water for their development and distinguish themselves from other aquatic insects by their unique shape.They primarily feed on organic debris, can be predatory or cannibalistic, and move in jerky motions **(Himmi et al., 1998)**.The mosquito larva uses its mandibular brushes (rotary palps) to feed by rapidly and regularly agitating, forming small tufts **(Robert, 2001).**

Culicids undergo four larval molts.The first three stages have variable chaetotaxic characteristics, making species identification difficult. In practice, the larval morphology most commonly described is that of the fourth stage **(EL AGBANI, 1995)**.

The head the structure consists of three chitinous plates: one dorsal, median, and unpaired called the fronto-clypeus, along with two lateral and symmetrical plates. The head is enclosed within a sclerotinized capsule **(OUAKID et al., 2008)**.The mouthparts are of the grinding type, characterized by mobile transversal mandibles composed of long curved setae, which serve a prehensile function. The antennae, inserted on the sides, are typically long and spatulate, bearing a tuft of setae widely employed in taxonomy**(EL AGBANI, 1995)**.

The thorax of Culicids is stout and devoid of appendages, theoretically subdivided into pro, meso, and metathorax. It appears as a slightly flattened spherical mass dorsoventrally, with groups of stiff hairs arranged in a fan-like pattern, serving as the only external indication of its segmentation**(Himmi et al., 1998)**.

The abdomen of Culicid larvae is more flexible than the thorax and consists of nine segments. The stigmatic openings are located on the dorsal part of the eighth segment, being sessile in *Anophelini* and opening at the end of a chitinous tube or siphon in *Culicini*. The ninth segment houses the complex system of anal hairs and two hyaline appendages framing the anal opening. The siphon, of variable length depending on the species, facilitates larval respiration **(Senevet and Quievreux, 1941)**.

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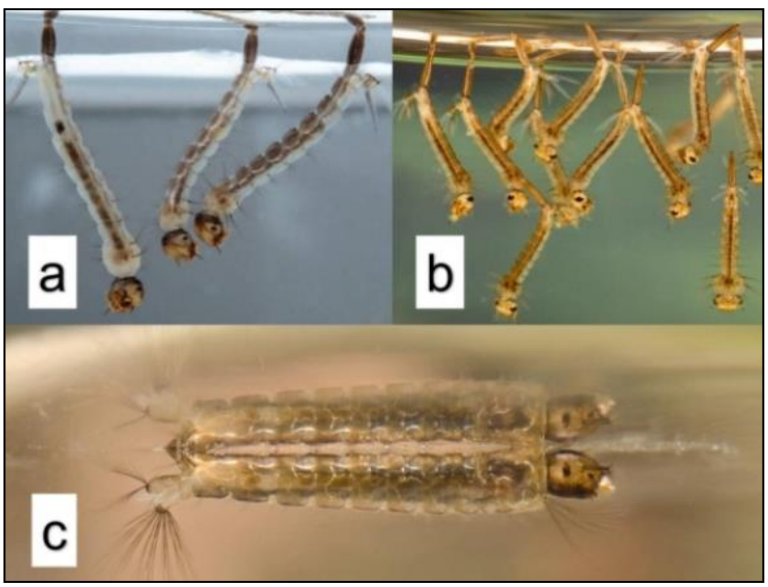

Figure 1: Some photos of Culicidae larvae: a – genus *Aedes*, b – genus *Culex*, c – genus *Anopheles* (Source : istockphoto.com, cdc.gov, aquaticinsect.net respectivement).

C) The nymph

The transformations enabling the mosquito to transition from the aquatic to the terrestrial environment begin at the end of larval development with the breakdown of muscles and continue in the pupa with the development of an entirely new system **(Senevet and Quievreux, 1941)**.The nymph stage is brief, not exceeding four days. The nymph has a comma-like shape and does not feed, instead drawing from the reserves accumulated during the larval stage **(Himmi et al., 1998)**.

D) The imago (adult)

The three fundamental parts of the mosquito's body are very distinct:

The head has a globular shape with two large, nearly contiguous compound eyes, often blue or metallic green. It possesses a pair of antennae with fifteen segments, feathery in males and almost smooth in females. Additionally, it has piercing-sucking mouthparts**(EL AGBANI, 1995)**.

The thorax results from the fusion of three rigid segments: the prothorax, the mesothorax, and the metathorax **(Rioux, 1958)**,the thorax is covered with elongated hairs. The second segment, the most developed, bears a pair of wings covered with numerous scales, with a relatively simple venation. A pair of halteres, small membranous scales, is also observed on this segment, considered as wing appendages. The third segment, less visible, lacks wings but is endowed with a rich nervous network; it appears to have a sensory function. It is crucial for flight: removing even a single haltere makes it impossible **(KHARCHI Roumaissa, 2023)**.

The abdomen its dorsal surface is uniformly dark or adorned with pale bands or triangles**(Senevet and Quievreux, 1941)**, the abdomen consists of ten segments, of which only eight are externally visible **(Rioux, 1958)**, male hypopygia are equipped with complex anatomical diverticula and are of significant interest in systematic studies **(Berchi, 2000)**. on the other hand, the female hypopygium has a relatively simple morphology but plays a taxonomic role in distinguishing between genera**(EL AGBANI, 1995)**.

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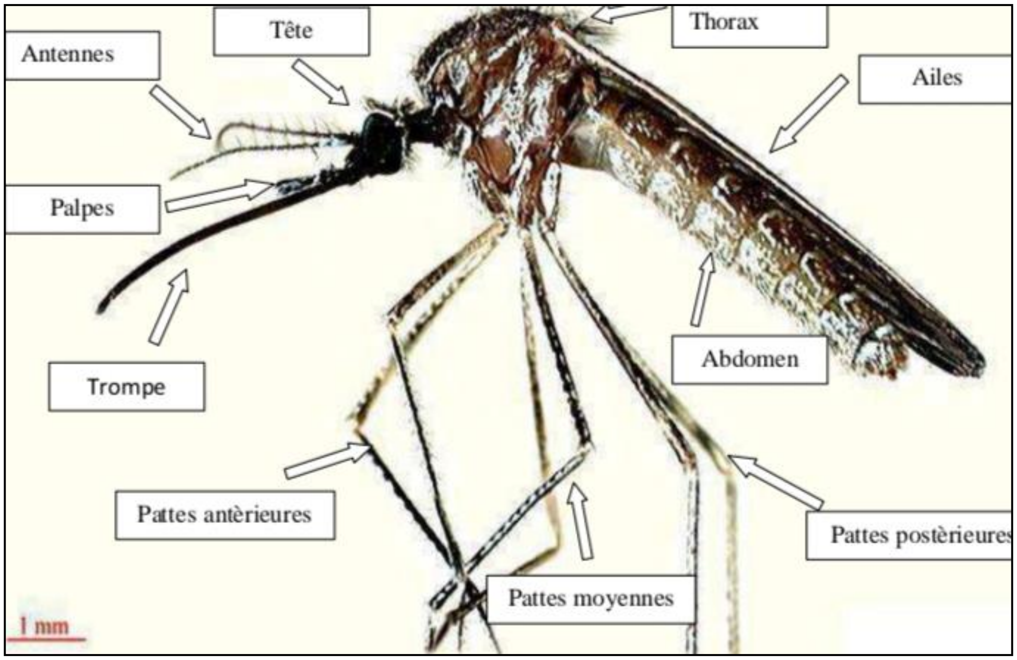

Figure 2:General morphology of an adult Culicinae(*Culex*) **(Brunhes, 1999)**.

5 Ecology and life cycle of mosquitoes

The mosquito development cycle spans from twelve (12) to twenty (20) days and involves four (4) distinct stages: egg, larva, nymph (or pupa), and adult. This transformation occurs in two phases: an aquatic phase and an aerial phase**(Adisso and Alia, 2005)**.Each species has well-defined ecological requirements**(Phetsouvanhet and Sidavong, 2003)**.

The aquatic phase begins a few days after fertilization. Depending on the species, eggs of various shapes (spindle-shaped, elongated, swollen in their environment, and sometimes equipped with tiny lateral floats) are laid by the female in different environments. Egg laying often ranges from 100 to 400 eggs, and the egg stage lasts for two to three days depending on environmental factors such as water temperature, pH, nature, and abundance of aquatic vegetation, as well as associated fauna. The size of an egg is approximately 0.5 mm **(Rodhain and Perez, 1985)**.

Upon reaching maturity, the eggs hatch, giving rise to first-stage larvae measuring 1 to 2 mm. these larvae, until they reach the fourth stage at 1.5 cm in length, primarily feed on organic matter, microorganisms, and sometimes even live prey in the case of predatory species. Despite etheir aquatic habitat, mosquito larvae have developed aerial respiration mechanisms facilitated by respiratory siphons or spiracles. The fourth-stage larva is easily visible to the naked eye due to its size, featuring a head with lateral compound eyes and two antennae, followed by the thorax and abdomen **(Kpondjo, 2008)**.

After a period of six (6) to ten (10) days or longer, influenced by water temperature and food availability, the fourth molt leads to the emergence of a nymph. This stage is known as pupation **(Guillaumot, 2005)**.

This passage outlines the numphal phase of the mosquito's life cycle. During this stage, the nymph, although mobile, abstains from feeding for one to five days. It surfaces intermittently to breathe and swiftly descends when disturbed. As this phase concludes, the nymph elongates, its exoskeleton splits dorsally, enabling the adult mosquito to emerge from the exuviae in a process known as emergence. Throughout this roughly fifteen-minute period, the insect remains vulnerable and defenseless against various surface predators **(Rodhain and Perez, 1985)**.

In the aerial phase, adult mosquitoes of both sexes mate either while flying or within vegetation, typically within a flight range of one to two kilometers. The males, aided by the long hairs on their antennae, can detect the buzzing sound generated by the rapid wing beats of the females as they approach swarms during mating flights. During this encounter, the male fertilizes the female by transferring a portion of his semen. The female then stores the male's semen in a globular ampoule or storage vesicle called the spermatheca, allowing her to mate only once**(DE MALADIES; Darriet, 1998)**.

Adult male and female mosquitoes primarily feed on sugary sap, nectar, and other plant secretions. However, after fertilization, females specifically seek out a blood meal to obtain the proteins and amino acids essential for egg maturation. This blood meal, acquired from a vertebrate such as a mammal, amphibian, or bird, is subsequently digested in a secluded location **(Guillaumot, 2005)**.

Upon becoming gravid, the female mosquito promptly seeks an appropriate site to deposit her eggs for larval development. Typically, egg-laying takes place at dusk, with the preferred habitat being stagnant or slow-moving water, whether it is freshwater or saline**(Ayitchedji, 1990)**.

asserts that blood, water, and a temperature of at least 18°C are the three necessary conditions for the reproduction and development of certain mosquitoes**(Iroko, 1994)**.

The female mosquito is generally larger than the male and has discrete antennae adorned with a small number of hairs. In contrast, the male's antennae are feathery, bushy, and equipped with long hairs. Unlike males, who often remain near the larval breeding site, blood-feeding females are highly mobile and frequently move towards human habitations**(Mobilization et al., 2003)**.

Adult mosquitoes can have close interactions with humans and their dwellings, which serve as refuges for them. Their activity hours vary depending on the species. Their seasonal frequency fluctuates according to changes in the environment and climate. Generally, the longevity of adults is short, ranging from 10 to 40 days, with females living longer than males. In temperate regions, some species can live on average up to 6 to 7months**(Merabti, 2016)**.

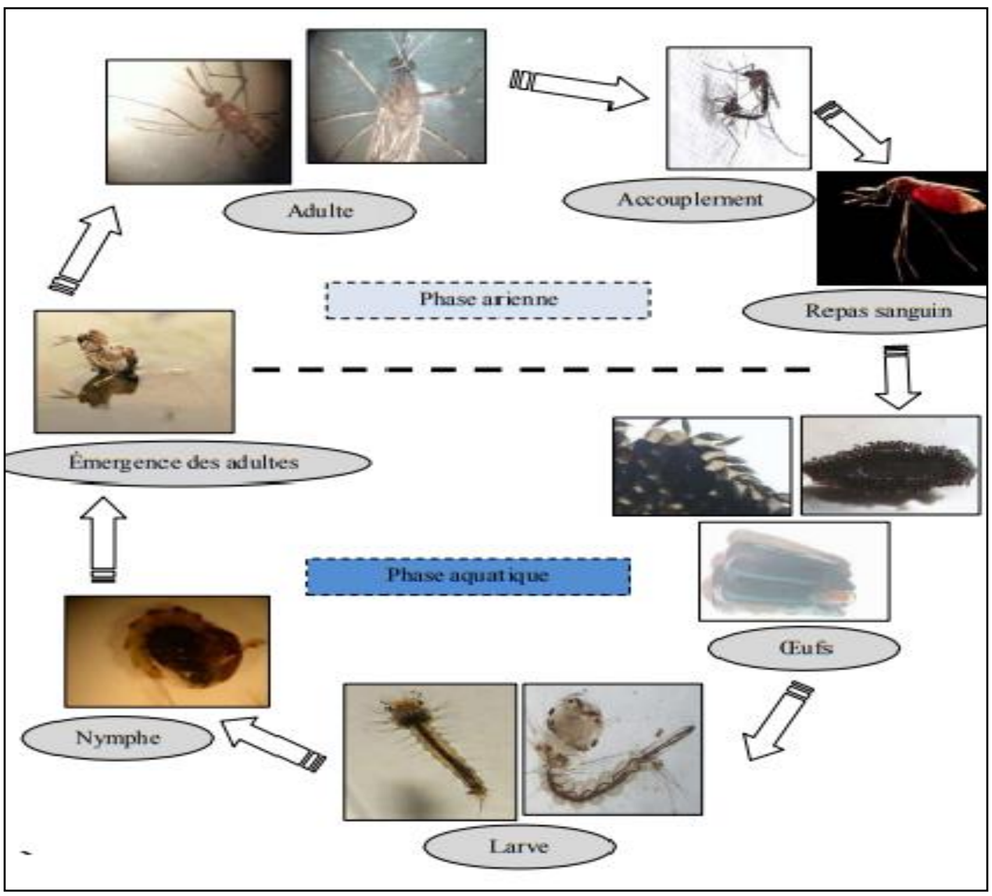

Figure 3: Mosquito life cycle**(Merabti, 2016)** .

6 Geographical distribution

The movement of goods , animals , and humans , as well as climate changes , increase the ability of mosquitoes to spread , whithout also forgetting the role of wind in this process **(Muja-Bajraktari et al., 2019)**.

7 Species to which the study was applied

The spread of *Ae. aegypti* between latitudes of 40 ° north and south and its being tropical mosquito **(Oecd, 2018)** and its transmission to all of zika virus , Dengue, **(Anoopkumar et al., 2017)** chikungunya virus , yellow fever virus **(Shannon et al., 1938)**and also the spread of *An. Stephensi* in many regions within East and Central Asia **(Surendran et al., 2019)** and its transmission to all of *Plasmodium .vivax*, *P. falciparum* in urban area**(mondiale de la Santé, 2019)** the geographical distribution of *Cx .pipiens*is widespread across the Northern Hemisphere, including North America, Europe, and parts of Asia**(Luande et al., 2020)** the key diseases that *Cx .pipiens* can transmit are filarial parasites**(Aung et al., 2023)**, Rift Valley Fever virus, and West Nile virus, with the potential to also transmit Japanese encephalitis virus and avian malaria**(Dehghan et al., 2013)**.Has made these species very popular among scientific research. This made many researchers try to suppress it using the sterilization methods.

chapter II METHODS

CHAPTER II METHODS

1 Sterility

Structural and functional inability to reproduce, complete or partial failure to produce functional gametes or viable fertilized eggs, or finally complete involuntary inability to reproduce .This is what biological dictionaries stipulate in difining infertility , as all of these changes cover either morphological , physiological, genetic, that ultimately lead to infertility **(Dyck et al., 2021)**.

2 Irradiation

A) Description

H. J. Muller's discovery of the mutagenic effects of ionizing radiation laid the foundation for the sterile insect technique (SIT). This technique, used by Kipling and his team following World War II, aimed at eradicating the screwworm fly in Central America, Mexico, and the USA. Since that time, many effective programs have been implemented, but they are used against many species. Much research was conducted on mosquitoes in the seventies. It is an environmentally friendly technology developed in the USA. Its aim is to reduce reproduction by exposing insects to radiation, thus increasing the rates of sterile mating. It is currently applied on 6 continents and has been used for six decades.**(Dyck et al., 2021)**.

B) Technical summary

The basic principal of mosquito sterilization technology that relies on radiation is :Exposing target species, which have been bred in large numbers, to gamma rays or increased X-rays to induce sexual infertility.**(Dyck et al., 2021)**or high-energy electrons.**(Helinski et al., 2009)**. This is done through a set of steps : which are mass breeding and radiation sterilization , and then finally releasing the largest number of males from that targeted area, which lead to the failure of human production when these male mate **(Wilke and Marrelli, 2012)**.

C) The used compounds

The ability to penetrate, availability, cost, safety, and, finally, relative biological effectiveness (RBE) are the factors that intervene in choosing the appropriate radiation**(Dyck et al., 2021)**the use of gamma rays is the most common, but this does not preclude the possibility of using X-rays and high-energy electrons.**(Helinski et al., 2009)**.

D) Method of influence

Radiation affects both gonadal and somatic cells. In the first case, it destroys the chromosomes, making the insect reproductively sterile. It causes fragmentation of the germ cells, leading to unbalanced gametes, resulting in the death of fertilized eggs and embryos. As for somatic cells, it causes effects such as a decrease in the ability to fly, reduced tendency to mate, shortened lifespan, and ultimately, the death of insects**(Dyck et al., 2021)**.

Ae. aegypti decrease fertility in males , a slight decrease in their life span and a decrease in the average number of eggs **(Bond et al., 2019)**.

Cx. pipiens noticeable effects on reproductive and biological activity **(Hasaballah, 2018)**.

An.stephensi reduce both of fertility and fecundity **(Yadav et al., 2010)**.

E) Advantages and disadvantages of technology

Sterilization of mosquitoes by radiation is technology that has a positive side represented by environmentally , species-specific, **(Wilke and Marrelli, 2012)**, overcoming many problems faced by other methods **(Giesbrecht, 2021)**and adult males do not transmit diseases **(Alphey et al., 2010)** and another negative side represented by both Sterile males must be placed in the right placed in the right place and time to do their job , and this is difficult , raising the selected individuals en masse , release must be precise , especially if the spread of the targeted mosquito is low **(Alphey et al., 2010)**and if the dose of radiation used increase , sterility increase , but competitive ability decrease **(Dyck et al., 2021)**.

3 Precision guided sterile insect technique pgSIT

A) Description

Rapid development and numerous applications characterize what technology has witnessed in the past decade. Where was find that pgSIT technology is basically based on CRISPR technology **(Ying et al., 2023)** and it is an effective and safe technology that combines the precision of genetic engineering with the benefits of traditional methods. It relies on releasing genetically sterile males to mate with females.**(Gendron et al., 2023)**.

B) Technical summary

The principle of technology precision-guided sterile insect technique is to kill females and make males sterile by using genetic engineering **(Gendron et al., 2023)**. This is what the pgSIT technology requires, two identical Strains. As one of them expresses guide RNAs targeting genes related to male fertility and female survival, while the other expresses Cas9 nuclease. When crossing between the two strains occurs, all resulting strains inherit guide RNAs and Cas9 nuclease. This enables CRISPR-based genome editing to occur, making males sterile and causing female offspring to die.**(Wang et al., 2024)**.

C) Method of influence

Disabling the genes responsible for male fertility and female survival is what the pgSIT technology relies on and accomplishes CRISPR technology **(Kandul et al., 2019)**

Aedes aegypti disabling essential genes that determine sex and fertility **(Li et al., 2024)**.

D) Advantages and disadvantages of technology

All technologies have two sides , one positive and the other negative .As for the positive aspect of technology it is represented by both :Males gain fitness and high competitive ability , an important role in space on disease vectors ,safe **(Kandul et al., 2019)**,releases are only for sterile males who will not introduce genetic matriel into the environment , reduced use of drugs and pesticides **(Grilli et al., 2021)** and while the negative is unknown effects on the environment, misuse ,un expected and completely unintended consequence**(Annas et al., 2021)**.

4 Chemosterilants

A) Description

Chemosterilants, chemical compounds with the capacity to induce sterility in organisms, particularly insects**(KS, 1964)**, have a rich history dating back to the 1960s and 1970s. During this era, thousands of potential chemosterilants were identified, with aziridine-based alkylating agents emerging as notably effective options for mosquitoes. Field trials employing chemosterilants like tepa and thiotepa successfully suppressed insect populations. However, concerns regarding toxicity and environmental impact eventually led to a decline in research and development efforts in this area. Nevertheless, recent years have witnessed a resurgence of interest in chemosterilants, spurred by advancements in genetics and molecular analysis of disease vectors **(Baxter, 2016)**.

B) Technical summary

The principle of using chemosterilants involves disrupting the reproductive capabilities of target species to reduce their numbers and mitigate the impact of pests on human health and agriculture to effectively use chemosterilants in pest management, several key steps must be followed **(KS, 1964)**.

Firstly, identify the target insect stage, then; select the appropriate chemosterilant considering factors like its mode of action and application method. Next, determine the optimal concentration needed to induce sterility in the target insect population and apply the chemosterilant using suitable methods, ensuring effective exposure. Monitor the treated population regularly, assessing parameters like mortality rates and population dynamics to gauge effectiveness. Consider environmental impact and minimize harm to non-target organisms. Repeat applications as needed, adhering strictly to safety guidelines throughout **(Baxter, 2016)**.

C) Basic materials

There are many compounds that cause infertility in the three species studied, and among these compounds is:

Apholate**(KS, 1964)**, tepa**(KS, 1964)**, and aphamide **(KS, 1964)**, Aster 20SP **(Abdelouaheb, 2016)**, thiotepa**(Gato et al., 2014)** ,bisazir**(Baxter, 2016)**, Amide **(Sharma et al., 1978)**

D) Method of influence

In *Culex pipiens*, chemosterilants can induce genetic damage to reproductive cells, thus disrupting normal reproductive functions such as sperm production, egg development, and mating behavior…ect**(Baxter, 2016)**.

Chemosterilants can affect an *Aedes aegypti* by causing chromosomal aberrations, genetic imbalances, and developmental abnormalities…ect**(KS, 1964)**.

It can affect an *Anopheles stephensi*by causing reduction in oviposition**(Sharma et al., 1978)**, the alkaloids caused morphological abnormalitiesin *Anopheles stephensi* such as larval-pupal intermediates and half-ecdysed adults……..ect**(Saxena et al., 1993)**.

E) Advantages and disadvantages of technology

Chemosterilants have both advantages and disadvantages in insect sterilization for vector control programs. On the positive side, they are cost-effective, historically proven effective, andmaintain competitiveness of sterilized males. However; concerns exist regarding environmental impact, regulatory challenges, and safety considerations. While chemosterilants offer a viable and successful method for insect sterilization, careful attention to environmental, regulatory, and safety aspects is crucial for their responsible use in vector control programs**(Gato et al., 2014)**.

5 Wolbachia induced cytoplasmic incompatibility

A) Description

The history of utilizing *Wolbachia* to sterilize mosquitoes traces back to its discovery in the reproductive tissues of the mosquito *Culex pipiens* in 1924 by Hertig and *Wolbachia*. Formally described as *Wolbachia pipientis* in 1936 by Hertig, *Wolbachia* has been found to manipulate mosquito reproduction through various means such as inducing cytoplasmic incompatibility (CI), parthenogenesis, feminization of males, and male killing in different host species. These unique characteristics have led to the development of *Wolbachia* as a novel strategy for controlling vector mosquitoes**(Kulkarni et al., 2019)**.

Wolbachia induces cytoplasmic incompatibility (CI) in mosquitoes, which is a form of reproductive manipulation resulting from *Wolbachia* infection. CI occurs when infected males are unable to successfully reproduce with uninfected females or females infected with a different *Wolbachia* strain, leading to reduced or no offspring production. This mechanism can be harnessed to sterilize mosquito populations, potentially reducing their ability to transmit diseases**(Bian et al., 2013)**.

B) Technical summary

The principle of using *Wolbachia* involves manipulating or eliminating the obligate endo symbiotic bacteria *Wolbachia*, which is required by arthropods for blood meal digestion, reproduction, or development. By targeting *Wolbachia*, arthropods can be sterilized, killed, or have their life cycles disrupted, leading to a reduction or elimination of populations. This strategy is considered to be potentially efficacious and cost-effective, with minimal non target effects**(Sakamoto and Rasgon, 2006)**.

The method of using *Wolbachia* to sterilize mosquitoes begins with the infection of male mosquitoes with *Wolbachia*. These infected males are then mass-reared in facilities to ensure a sufficient population for subsequent release into the environment. Upon release, these infected males mate with wild type females, transferring *Wolbachia* to them. This mating process leads to the production of non-viable eggs, ultimately suppressing the population of wild type mosquitoes over time. This approach is accompanied by rigorous monitoring and evaluation to assess its effectiveness and make necessary adjustments**(Lim et al., 2024)**.

C) Basic materials

The basic material in the technique of sterilizing mosquitoes using *Wolbachia* is the cytoplasmic incompatibility (CI) phenomenon. This phenomenon effectively sterilizes uninfected females when they mate with infected males, as the sperm of the infected male is unable to form viable offspring during the egg fertilization process**(Florez et al., 2023)**.

D) Method of influence

The *Wolbachia* infection in *Culex pipiens* causes cytoplasmic incompatibility (CI), which leads to a reduction in egg-hatch frequencies**(Yang et al., 2021)**.

The *Wolbachia* in *Aedes aegypti* (wMelBr strain) resulted in a decrease in fecundity and egg viability compared to mosquitoes without the bacterium, however, the average number of eggs laid per female with *Wolbachia* was similar **(Farnesi et al., 2021)**.

Wolbachia's effects on *Anopheles stephensi* include immune activation, decreased fertility, pathogen resistance, and disruption of pathogen development **(Epis et al., 2020)**.

E) Advantages and disadvantages of technology

Wolbachia based mosquito control offers several advantages. Firstly, it can effectively sterilize mosquitoes, thus suppressing population growth. leading to fewer instances of diseases transmitted by them, however, there are notable disadvantages to consider, one significant concern is the potential environmental impact, introducing *Wolbachia* infected mosquitoes into ecosystems may disrupt non-target species and alter the dynamics of the environment,ethical considerations also come into play, deliberately releasing genetically modified organisms raises ethical questions about our responsibility to the environment and other species, furthermore, there's a risk of resistance developing over time. Mosquito populations could evolve to become resistant to *Wolbachia* induced cytoplasmic incompatibility, undermining the long-term effectiveness of this strategy **(Xie et al., 2016)**.

chapter III METHODOLOGY

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1 Morphological identification

A) Morphological identification of larvae

In the larval stage, mosquitoes go through four molts, and we cannot determine the species in molts 1, 2 and 3 because they show different characteristics of the chaetotaxy. Therefore, the only one that allows determining the morphology of the species is molts 4 **(Merabti, 2016)**.

- ***Aedes aegypti***

The first steps was to obtain larvae from a laboratory culture in this case 5 individuals from each larval stage L1,L2,L3,L4 were used and they were fixed in 2.5% glutaraldehyde and then in osmium tetraoxide and they dried through critical point dried, ion sputtered gold. As for examination, a scanning electron microscope(SEM) of the type (Hitachi S-570).In this study , they focused on the siphon , segments VIII and X , , head capsule and the mouth brushes.

They noticed that in the third and fourth larval instars, the lateral palatal brushes appeared in the form of complex and scattered clumps, which made it difficult to distinguish the plate, and the latter was wide.

The ventral brush in the segment X of the fourth larval instars was composed of four pairs of strong bristles. As for the comb scales , their number and development increased from the first to the fourth instars, because the comb scales of the later instars showed one long median denticulate with a slightly curved and more prominent apex than the others and had a varying number of subapical denticles where they were from 10 to 14subapical denticles in the fourth instar**(Schaper and Hernández-Chavarría, 2006)**.

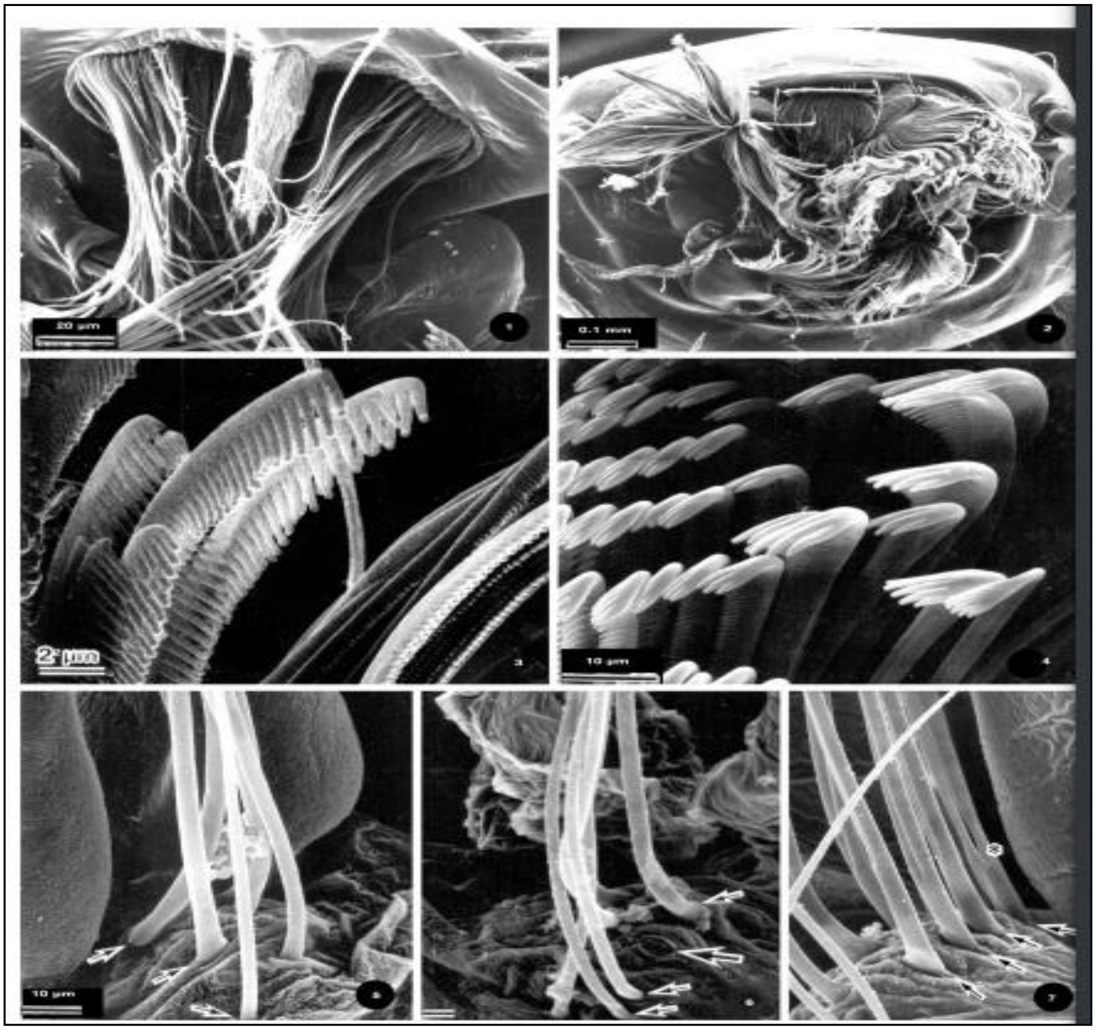


Figure 4: 1_2Frontal views of mouth brushes at the 1st and 4th instars 3_4. Lateral palatal filaments of the 1st and 4th instars 5_7Ventral brush of segment X of the 2nd, 3rd and 4th instars, respectively **(Schaper and Hernández-Chavarría, 2006)**.

- ***Culex pipiens*** The larvae were collected using a plastic dipper with a capacity of 350 ml and the larvae were identified using the taxonomic identification key.

The first steps were fixation with 0.1 buffer from 2.5% glutaraldehyde with pH = 7, 4 for three days. This was to preserve the structure of the sample and ensure the solidity of the tissue .Secondly, drying by passing the larvae through a series of increasing concentrations of ethanol for 5 min for each concentration to remove water. Then they were also placed in 100% ethanol. Thirdly, they were fixed on a special holder with a double-sided carbon tape connected to electricity, and finally, they were plated with gold. To allow it to be seen with a scanning electron microscope was its type 450 FEI Nava Nano SEM and the study was done on the abdominal segments (VIII+IX) and siphon.

Result pectin teeth contain a group of teeth ranging in length from 61.26 -to 64.62 µm, and each one of them is wide at the base and has five teeth. As for the comb scales, they are in irregular rows and the length of each one is 26.02 µm and is surrounded by many teeth smooth , as for the lateral palatal brush filaments, they have a broom-like structure with many simple teeth. Finally, there are fine spines arranged on the surface of the siphon in the form of rows of variable lengths and have the shape of fish shells **(Abed and Kareem, 2022)**.

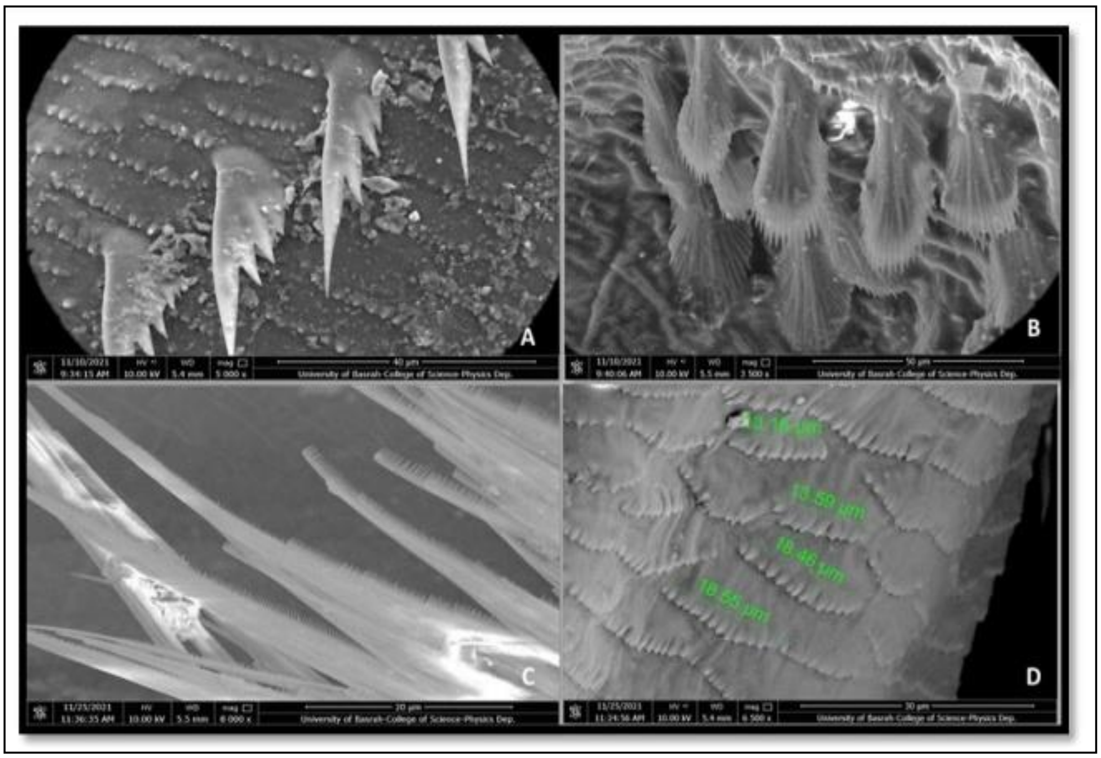


Figure 5: Scanning electron microscope of *Cx. pipiens* larva, A- Pectin teeth, B- Comb scale, C- Lateral palatine brush filaments, D- Microspine patterns**(Abed and Kareem, 2022)**.

- ***Anopheles stephensi*** After collecting larvae from the rice fields , they were killed using boiling water , then stored in 70% ethanol , then dried in graded series of alcohol , then passed in Critical Dry Point then they fixed on SEM specimen stubs using a small adhesive tape on the sides , painted with gold and scanned with SEM type of JSM- 6100.

As for the parts of the mouth , they noticed that it consists of Anteromedian Palatal Brushes (APBr), Mandibular Brushes (MnB), Lateral Palatal Brushes (LPB) Maxillary Brushes (MxB)… and for the Antenna it is straight and short and has three types of sensilla: Sensilla Trichodea Blunt (STB), Sensilla Basiconica (SB) and Sensilla Trichodea Pointed (STP) , as for Nuttall & Shipley's Organ (NSG) it is what distinguishes the Anopheline larvae from others and it consists of a bilobed membranous structure borne dorsally on each side of pro-thorax, holds the thorax to water surface during feeding and finally the Mentum (Mt) has four teeth . On both sides of the middle tooth , the first tooth is round and short , while the remaining three teeth are approximately equal in distance **(Kirti, 2014)**.

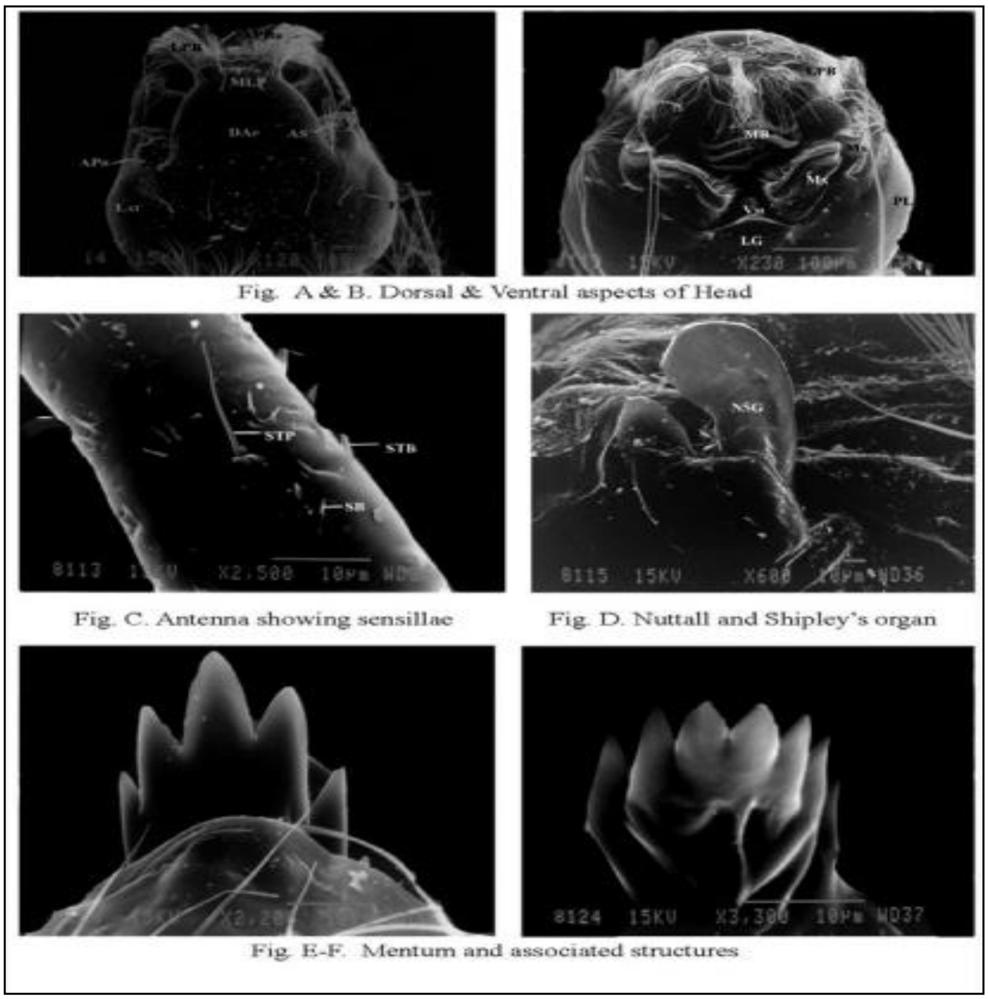


Figure 6 : the appearance of the larval stage of a *Anopheles stephensi* mosquito with a scanning electron microscope**(Kirti, 2014)** .

B) Morphological identification of adult

After placing this mosquito under the microscope**(Merabti, 2016)**

- ***Aedes eagypti*** *Aedes aegypti* mosquitoes, typically 4-7mm in length **(Yeap et al., 2013)**, exhibit distinct characteristics, females are larger than males, they have a dark brown/black body with white leg markings and a lyre-shaped pattern of silver scales on the thorax, their proboscis is short, abdomen may have white bands, and wings bear dark/light scales forming a pattern, these features aid in their identification **(Rueda, 2004)**.
- ***Culex pipiens*** Morphological identification of *Culex pipiens* encompasses various features: plumose antennae denote females; equal maxillary palp length to proboscis is key; absence of specific setae excludes other genera, labium without a ring, thorax ornamentation, setae observation, tibia coloration, and wing structure positions are distinguishing factors, these characteristics collectively differentiate *Culex pipiens* from similar species **(Merabti, 2016)**
- ***Anopheles stephensi*** To identify *Anopheles stephensi*, measure the mosquito's body length under a microscope, if it's less than 3 mm, check the proboscis length, if longer than the head and thorax combined, examine wing vein patterns for a distinct dark and light scale pattern, confirm by inspecting the abdominal coloration, which should be predominantly dark with light bands or spots, molecular techniques may also be used for accurate identification**(Hawaria et al., 2023)**.

2 Hunting method

- The hunting method for mosquitoes involved the use of Gravitraps, Gravitraps are devices designed to attract and capture adult female mosquitoes, these traps were strategically placed in public spaces along corridors in high-rise housing estates, the traps were evenly distributed throughout the apartment blocks, with approximately one trap for every 20 households **(Consortium and Ching, 2021)**.

Figure 7: Picture of a Gravitrap with mosquitoes trapped on the sticky lining**(Ong et al., 2020)**.

- The hunting method involves using a sound attraction assay, this assay plays a 10-second 600 Hz sine tone on one side of a cage to mimic female flight tones, attracting male mosquitoes, to measure their response, the number of mosquitoes landing around the speaker area is quantified at 5-second intervals throughout the stimulus **(Li, Yang *et al*. 2021)**
- The use of ovitraps as a method for monitoring mosquito populations, Ovitraps are black 300 mL plastic cups lined with filter paper and filled with tap water, the filter papers are collected weekly, and the eggs are allowed to mature under wet conditions for three days before being dried, counted, and classified as either field-hatched or non-hatched eggs, this method allows for monitoring the frequency and density of the eggs **(Gato et al.).**

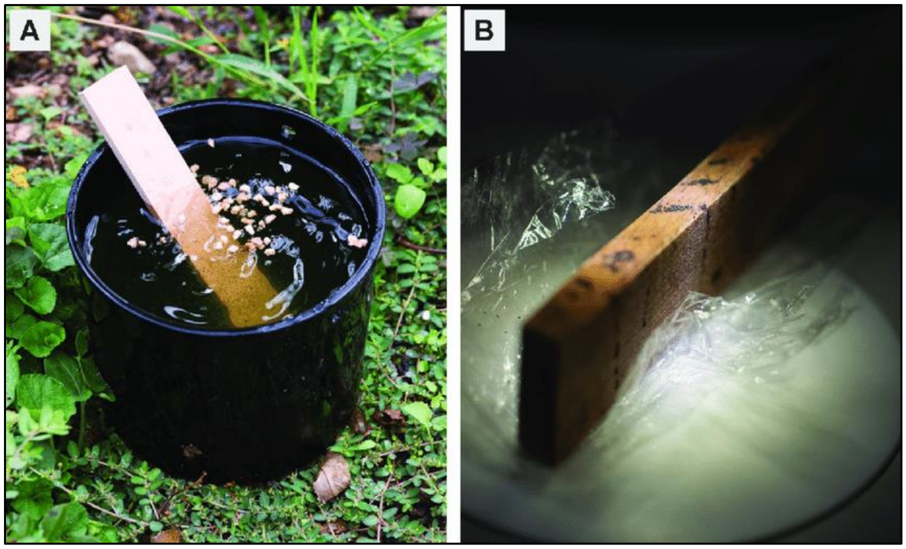


Figure 8:Ovitrap used to collect eggs of invasive *Aedes species*. (A) The female mosquitoes glue their eggs on the slat that is plunged into the water inside the flower pot. the slats were collected and inspected biweekly.(B) Slat with *Aedes* mosquito eggs**(Müller et al., 2020)**.

3 Conservation and breeding

After obtaining approval from ethics committees in research and biosecurity **(Bond et al., 2019)**,we first acquire mosquitoes from colony laboratories **(Yadav et al., 2010)** or medical insect research centers, where the mosquitoes are raised in special laboratory conditions:

1) Humidity 70 ± 10% **(Hassan et al., 2017)** rh or 80 ± 5% rh **(Bond et al., 2019)**.
2) Temperature 22-28°C **(Ernawan et al., 2018)** or 28 ± 2˚C **(Bond et al., 2019)**.
3) 12–12 h light-dark regimen **(Hasaballah, 2018)** or 14 h:10 h (light: dark) , Where adults feed on a solution of sucrose and females obtain meals from blood **(Bond et al., 2019)**.

4 Application of methods

A) Irradiation with γ-RAY

They irradiated Mosquito pupae **(Gato et al., 2021)** or adults one hour old **(Hasaballah, 2018)** using one of the gamma ray irradiation devices , including Gamma cell-40 (cesium-137) irradiator **(Hassan et al., 2017)** the 60Co source (Gamma - 5000 irradiator, BRIT, BARC, Trombay)**(Hasaballah, 2018)**.

Irradiation methods differed from one article to another according to the needs of each researcher , as we find an example in the first article**(Hassan et al., 2017)**that studied effect of both doses of gamma rays (LD25 - 23 Gy), (LD50 - 41Gy), (LD75 - 75 Gy) and (LD90 - 128Gy) on male mosquito pupae after exposing them to it , he waited for them to reach puberty , then allowed the irradiated adult males to mate with non-irradiated females to obtain the first generation F1 ,then after a complete life cycle , he allowed the resulting irradiated males from the first generation are also mated with non-irradiated females to obtain the second and third generation F3with the same way **.** Each dose was repeated 3 times across the three generations, 10 irradiated males were mated with 10 non-irradiated females. The treated males from each stage were then transferred with the healthy females to wooden cages (20 20 20 cm) and were fed for 3 days with a sugar solution 10% and starved on the fourth day, while on the fifth day, a blood meal was provided to females and she was allowed to lay eggs in dechlorinated water. The number of eggs was counted and divided into hatched.

nonhatched → embryonated

→ non-embryonated

it was calculated the sterility % and egg-hatchability formulation then they obtained the testicles from the males after killing them, put them in glutaraldehyde 5% and observed them with an electron microscope for the second article **(Hasaballah, 2018)** , two groups of pupae were obtained . For the first group, these pupae were collected one day before irradiation and were at the age of 24–30 h and were exposed to 3 doses of radiation 40Gy and 60 Gy or 80 Gy of gamma rays at the rate of each radiation dose 2.3 Gy/min and as for the second group they were not irradiated and then after emergence. Males and females were collected from the irradiated and non-irradiated group, and the ability to hatch eggs was calculated , and the fertility rate and infertility index were determined, the abdomens of irradiated females were obtained after 72h providing them with a blood meal, and they were fixed and then photographed with electron microscope regarding.

The third article**(Ernawan et al., 2022)**, irradiation was applied to both males adults who were the age of 1and 3 days and male pupae 1days where as for the adults , they were irradiated after being placed in ventilated and transparent plastic tubes (10 cm high, 8 cm diameter) in doses 20 Gy , 40 Gy , 60 Gy , 70 Gy , 80 Gy , and 100 Gy . As for pupae, they were placed in (3 cm high, 14 cm diameter) transparent tubes and we removed them. Excess water to keep them only moist, as for irradiation dose, they was the same the treatment was repeated 3 times and the rate of emergence, induced sterility, mating competitiveness and longevity were calculated in the fourth article **(Ernawan et al., 2018)** they have been using completely Randomized Design (CRD) at one level in this case a sterile male , they sterile male at age 1 to 5 day with dose 70 Gy and brought females and male non irradiated and allowed them all to mate , then they counted the number of eggs. In the fifth article, **(Bond et al., 2019)** both female and male mosquito pupae were exposed before 24–36 h puberty to 15, 30, 50,70 and 90 Gy radiation, and to evaluate the effect of radiation on fertility. 20 non irradiated virgin females were brought to mate with 29 irradiated males and they were placed in cages (30x30x30 cm) and given a 10% sucrose solution. Starting from the fourth day, a single meal of blood was provided for three consecutive days using a membrane feeding system, after that, each female was placed alone in a centrifuge tube with in capacity of 50 ml containing 10 ml of deionized water and a strip of filter paper to place their eggs determine the total number of eggs laid and the number of eggs that hatch out of the total number of eggs produced. To determine the effect of this radiation on males, the experiment was repeated on 20 irradiated males and 20 non-irradiated females.

In the sixth article **(Gato et al., 2021)** , mosquito pupae were irradiated approximately 30 hours before puberty to reduce physical damage with a of 80 Gy was applied with a dose rate of 8 kGy/h. The irradiation packages consist of plastic tubes in a cylindrical shape. Where 6000 pupae was placed in each of the three tubes, without water, vertically. In the radiation room, after radiation returned to the culture-flasks to wait for it to reach maturity. Cardboard boxes were used and placed horizontally to serve as a container for adults, and ventilation holes were made in them, and a large opening allowed the neck of the flasks containing the pupae to be inserted. After all had departed the adults were placed in the boxes. The vial was covered with a plastic tube containing honey. The adults were also provided with cotton pads that had been moistened with a 10% honey solution and dechlorinated water release rates in the beginning, the release was for 40,000 sterile male for 3 consecutive weeks, then it increased to 50,000 for 3 weeks and, 60,000 for 5 weeks and 70,000 for two weeks and 80,000 sterile males for 5 weeks then it decreases to 60,000 for one weeks and to 50,000 in two weeks due to the lack of productivity. The monitoring was done using a network of eggs traps before the weekly release for two weeks, sterile adults males 2 days old, were collected and frozen and then placed in containers containing powder marked so that they adhere to their bodies when rotated . These mosquitoes were transferred to iron cages that were easy to open from the top to ensure ease of releasing the mosquitoes were in the field. The mosquitoes were provided with water and honey, then about 10,000 marked mosquitoes were released into “El Cano” community and Arroyo Arenas, and they were monitored through 21 traps containing bait spread over different dimensions. Then the mosquitoes found in the trap were collected and transported to the laboratory, killed, and their sex and specie were determined. The average life expectancy of sterile males and population density were calculated. Wild mosquitoes and evaluation of the flight behavior of released males according to the distance traveled in the seventh article **(Yadav et al., 2010)** , they use Male pharate (pupae 24 h before mergence) adults their sex was determined using a microscope, then they were placed in wet cotton in petri dishes , and they were transferred to the radiation source (The dose rate of radiation was approximately 2.37 KGy/h. Seventy and 100 Gy) inside ice box . After irradiation , the pupae were kept in cages and divided into 3 groups semi-emerged , non-emerged and emerged adults the emerging mosquitoes were fed by pieces of cotton soaked in 10% glucose solution to divide fertility in irradiated female pharate stluda with non-irradiated . The adults were collected in cages and provided with the same previous solution with raisins and placed according to 1:1 ratio in the cages. Each treatment was repeated 3 times in each cage that was presented one egg bowl filled with water and lined with wet filter paper was offered two days after blood meal, the eggs were collected daily and preserved for hatching . To calculate the egg hatchability by calculating the number of (L1) and fertility was calculated by dividing the number of eggs produced by the number of lives females.

B) Precision_guided sterile insect technique

Almost the same method of work included all of articles 1, **(Li et al., 2021)** and 2 **(Li et al., 2024)** and with some differences , including choosing the target gene or the number used , as after obtaining the mosquito the first steps was Guide RNA design and testing as the design of gRNAs targets both of *myosin heavy chain* (myo-fem, AAEL005656 and *β-Tubulin 85D* (βTub, AAEL019894) in both articles 1, but 2 used *Intersex* (ix, AAEL010217) genes and *doublesex* (dsx, AAEL009114)as for the stage Construct molecular design and assembly in which the manufactured parts (gRNAs) were introduced into plasmids then the third stage Generation of transgenic lines in articles1, as for the 2 it was named Engineering transgenic strains it was created by microinjecting preblastoderm stage embryos and plasmid ,then came the evaluation stage Genetic testing of established lines in the articles 1, and it was titeled in article 2 Genetic assessment of gRNA strains they crossed hemizygous mosquitoes that carry gRNAs strain with homozygous mosquitoes that carry Cas 9 and produced trans-heterozygous F1 progeny for. They tested them and allowed the resulting individuals to cross with WT then a blood meal was provided to them and eggs were collected and hatched to calculate the percentage of eggs obtained and calculate the survival rates from larva to adults articles1and2.

C) Chemosterilants

Through articles 17 and 18, the method of applying chemosterilant involves treating two-day-old larvae of mosquitoes with a chemosterilant solution, such as apholate or thiotepa, at a specific concentration like 15 ppm, this concentration induces almost complete female infecundity by inhibiting ovarian development, leading to reduced fecundity, the treated larvae eventually pupate, with males continuing to produce motile sperm, the chemosterilant ensures complete and irreversible sterility in adults.

D) Wolbachia induced cytoplasmic incompatibility

Through articles 11 and 14, the method of applying the *Wolbachia* technique involves releasing *Wolbachia* bacteria infected male mosquitoes into the target population, these males mate with wild-type females, leading to cytoplasmic incompatibility (CI) and the production of non-viable eggs, this approach aims to suppress vector populations and reduce disease transmission, the *Wolbachia* technique can be used in conjunction with traditional vector control methods.

chapter IV RESULTS AND DISCUSSION

Results and discussion

1 Irradiation with γ- RAY

- ***Culex pipiens***

In both the first and second articles, the study was related to *Culex pipiens* mosquitoes we found in the first study**(Hassan et al., 2017)** hatchability % and fecundity (number of eggs laid) were different for the first and the second generation F1,F2 than that untreated controls , as for the third generation F3 , there was no clear difference at untreated controls . They noticed higher levels of infertility (sterility index) in F1, F2 untreated controls as for F3, they almost sterility has been recorded. Irradiation has had an effect on growth areas (spermatocytes ,spermatids and spermatogonia) resulting in decreased reproductive ability**(Hassan et al., 2017)**.

Table 1 : The reproductive potential of males *Culex pipiens* resulted from irradiated pupae in the F1 generation**(Hassan et al., 2017)**.

Dose (Gy)	**No.of eggs laid/ten female (mean ± SD)**	**No. of hatched eggs (mean ± SD)**		**No. of non - hatching eggs (mean ± SD)**					**Sterility Index (S.I.) %**
				Total	**Embryonated**		**Non-Embryonated**		
		Total	**%**		**No.**	**%**	**No.**	**%**	
LD25 (23 Gy)	591.7 ± 16.9**	429.33 ± 13.01**	72.6	162.33 ± 4.72**	77.66 ± 2.08**	47.8	84.66 ± 5.03**	52.2	45.5
LD50 (41 Gy)	446.0 ± 32.**	229.66 ± 16.4**	51.5	216.33 ± 16.07**	117 ± 7.93**	54.1	99.33 ± 9.01**	45.9	70.8
LD75 (75 Gy)	347.7 ± 22.4**	80 ± 14.7**	23	267.66 ± 7.63**	185 ± 5.0**	69.1	82.66 ± 11.54**	30.9	89.8
LD90 (90 Gy)	_	_	_	_	_	_	_	_	_
Control	838.0 ± 7.2	788 ± 2.0	94	50 ± 8.71	8.33 ± 2.08	16.6	41.66 ± 6.65	83.4	_

(*) ¼ Significant < 0.05, (**) ¼ highly Significant, P < 0.01, ns ¼ non-significant > 0.05, SD ¼ standard deviation and (LD90) ¼ (_) at this dose the irradiated pupae did not emerge

From pupal stage.

Table 2 : The reproductive potential of males *Culex pipiens* resulted from irradiated pupae in the F2 generation**(Hassan et al., 2017)**.

Dose (Gy)	No.of eggs laid/ten female (mean ± SD)	No. of hatched eggs (mean ± SD)		No. of non - hatching eggs (mean ± SD)					Sterility Index (S.I.) %
				Total	Embryonated		Non-Embryonated		
		Total	%		No.	%	No.	%	
LD25 (23 Gy)	680.0 ± 26.6**	588.3 ± 19.5**	86.5	91.7 ± 9.8**	31.7 ± 3.1**	34.6	60.0 ± 7.0**	65.4	22.5
LD50 (41 Gy)	576.7 ± 23.**	446.0 ± 33.1**	77.3	130.7 ± 21.**	62.3 ± 11.0**	47.7	68.3 ± 10.**	52.3	41.2
LD75 (75 Gy)	527.3 ± 18.**	322.3 ± 18.**	_	205.0 ± 10.0**	100.0 ± 5.**	48.8	105.0 ± 5.0**	51.2	57.5
LD90 (90 Gy)	_	_	_	_	_	_	_	_	_
Control	797.0 ± 51.2	758.7 ± 45.0	95.2	38.3 ± 6.5	8.0 ± 1.0	20.9	30.3 ± 5.5	79.1	_

Table 3: The reproductive potential of males *Culex pipiens* resulted from irradiated pupae in the F3 generation**(Hassan et al., 2017)**.

Dose (Gy)	No.of eggs laid/ten female (mean ± SD)	No. of hatched eggs (mean ± SD)		No. of non - hatching eggs (mean ± SD)					Sterility Index (S.I.) %
				Total	Embryonated		Non-Embryonated		
		Total	%		No.	%	No.	%	
LD25 (23 Gy)	770 ± 44.44 ns	728.33 ± 49.07 ns	94.6	41.66 ± 11.54 ns	8.33 ± 2.88 ns	20	33.33 ± 10.40 ns	80	2.6
LD50 (41 Gy)	806.66 ± 45.09 ns	768.33 ± 37.52	95.2	38.33 ± 7.63 ns	7.33 ± 2.51 ns	19.1	31 ± 5.29 ns	80.9	2.6

		ns							
LD75 (75 Gy)	785 ± 37.74 ns	753.33 ± 33.29 ns	95.9	41.66 ± 7.63 ns	8 ± 1 ns	19.2	33.66 ± 7.02 ns	80.8	0.6
LD90 (90 Gy)	–	–	–	–	–	–	–	–	–
Control	790 ± 36.05	748.33 ± 32.53	94.7	41.66 ± 7.63	6 ± 1	14.4	35.66 ± 6.65	85.6	_

The processes of Spermatogenesis occur either in pupal stage and their irradiation leads to many tissue damages. Radiation has effect on normal cells, especially those that are rapidly growing , and in this case the sperm cells that turn into sperm because they are very sensitive to radiation, as their irradiation leads to the production of non–functional sperm or a decrease in their production, which leads to a decrease in reproductive ability **(Hassan et al., 2017)**.

The second article **(Hasaballah, 2018)** they founda noticeable decrease in the number of eggs a decrease in the fertility of females that mated with irradiated males as the rate of radiation increased and a decrease in the hatching rate and finally a histological examination of the ovaries of irradiated females in the pupal stage 72h showed a significant decrease in the number of eggs.

Table 4 : The effect of gamma irradiation on fecundity, egg-hatchability and sterility of F1 progeny resulted from *Cx. pipiens* irradiated pupae **(Hasaballah, 2018)**.

Dose (Gy)	**Mean ± SD**		**Sterility index%**
	Fecundity/25female	**Hatchability%**	
LD20 (40 Gy)	1575 ± 6.3**	64.5 ± 2.5**	78.5
LD30 (60 Gy)	1133 ± 11.0**	44.9 ± 1.5**	88.9
LD50 (80 Gy)	675 ± 5.6**	16.0 ± 0.6**	96.1

The pupae is considered the best stage for irradiation , and since irradiating small pupae causes a physical damage, it is better to irradiated pupae that are older. The decrease in fertility may be the result of changes that occurred at the level of the developing follicles that are present in the ovary **(Hasaballah, 2018)**.

In the second article **(Hasaballah, 2018)** , we found that the rate of sterility were at 78,5% at 40 Gy and 88,9% at 60Gy and 96,1 % at 80 Gy, these result of sterilizing of the pupae . As they did in the first article **(Hassan et al., 2017)** , the sterilized pupae were also used as a molar stage and then waited for them to reach maturity . It was noted that the rate of infertility decrease over the generations, including that we find that the best sterilization dose was 80Gy as it caused infertility in a percentage of 96, 1% and that infertility decrease with the passage of generations upon irradiation.

- ***Aedes aegypti***

About this species, there are four studies (3, 4, 5, and 6) article that talked about sterilizing *Aedes aegypti* mosquito by irradiation. The results of the first study (article 3. **(Ernawan et al., 2022)**, were as follows increased induced sterility is associated with increased irradiation doses and conversely high doses cause decreased mating competitiveness. They found that the best dose was 70 Gee as it caused induced sterility by 98% and slight decrease in the competitiveness index.

Table 5: Induced sterility (IS) and mating competitiveness (C index) of male *Age. aegypti* post irradiation at the different developmental stage/age at various doses **(Ernawan et al., 2022)** .

Developmental stage/age	**Dose (Gy)**	**IS (%)*a***	**C index*a***
Pupal stage	20	74.76 ± 1.95a	0.75 ± 0.10a
	40	86.19 ± 0.96b	0.68 ± 0.01ab
	60	95.70 ± 0.91c	0.47 ± 0.04bc
	70	98.72 ± 0.52c	0.32 ± 0.02cd
	80	100.00 ± 0.00c	0.21 ± 0.03d

	100	99.94 ± 0.03c	0.11 ± 0.01d
Adult stage aged 1 d	20	76.49 ± 1.65a	0.75 ± 0.04a
	40	87.39 ± 1.99b	0.70 ± 0.06ab
	60	89.32 ± 2.01b	0.57 ± 0.02b
	70	98.01 ± 0.73c	0.35 ± 0.03c
	80	99.95 ± 0.03c	0.22 ± 0.03cd
	100	100.00 ± 0.00c	0.14 ± 0.02d
Adult stage aged 3 d	20	56.76 ± 1.29a	0.71 ± 0.04a
	40	65.81 ± 0.35b	0.58 ± 0.07ab
	60	78.37 ± 0.61c	0.43 ± 0.03bc
	70	94.46 ± 1.14d	0.33 ± 0.01cd
	80	99.90 ± 0.05e	0.24 ± 0.01de
	100	100.00 ± 0.00e	0.15 ± 0.02e

***a*Data presented as mean ± SE. Data values followed with the same letter indicate no significant difference (*P* < 0.05) by one-way ANOVA and Tukey's Post hoc test.**

Partial or complete failure in producing zygote or gametes due to a dominant lethal mutation resulting from irradiation with gamma rays, this is what is called induced sterilitydamage to the somatic cells is what causes the disruption of the mosquito's development, and thus its competitive ability will be affected. The last stages of growth are considered the greatest resistance to irradiation**(Ernawan et al., 2022)**.

The second studies (fourth article**(Ernawan et al., 2018)**),in this expirement , there was an ratio of 9:1 meaning 9 sterile males versus one non_ irradiated male. This helped reduce the reproductive rate to 90% we can observe the sterility rate through the number of un-hatched eggs, as this percentage was more 90% in most mating group, except for the last group it was 86.91%. The fertility can also be observed by knowing the eggs that have turned into larvae, and since the highest percentage was in group 5 and lowest percentage was in group 2, then fertility does not decrease after a period of time after irradiation.

The decrease in fertility indicates that rate of competition between sterile males for non-irradiated males, it decreased due to cellular damage caused by gamma rays, as these rays are electromagnetic energy that a high invention potentiel.When tissues are exposed to them, the structural units of the cells will ionize, resulting in the the formation of reactive free radicals that can cause damage **(Ernawan et al., 2018)**.

The third studies (article 5) **(Bond et al., 2019)**

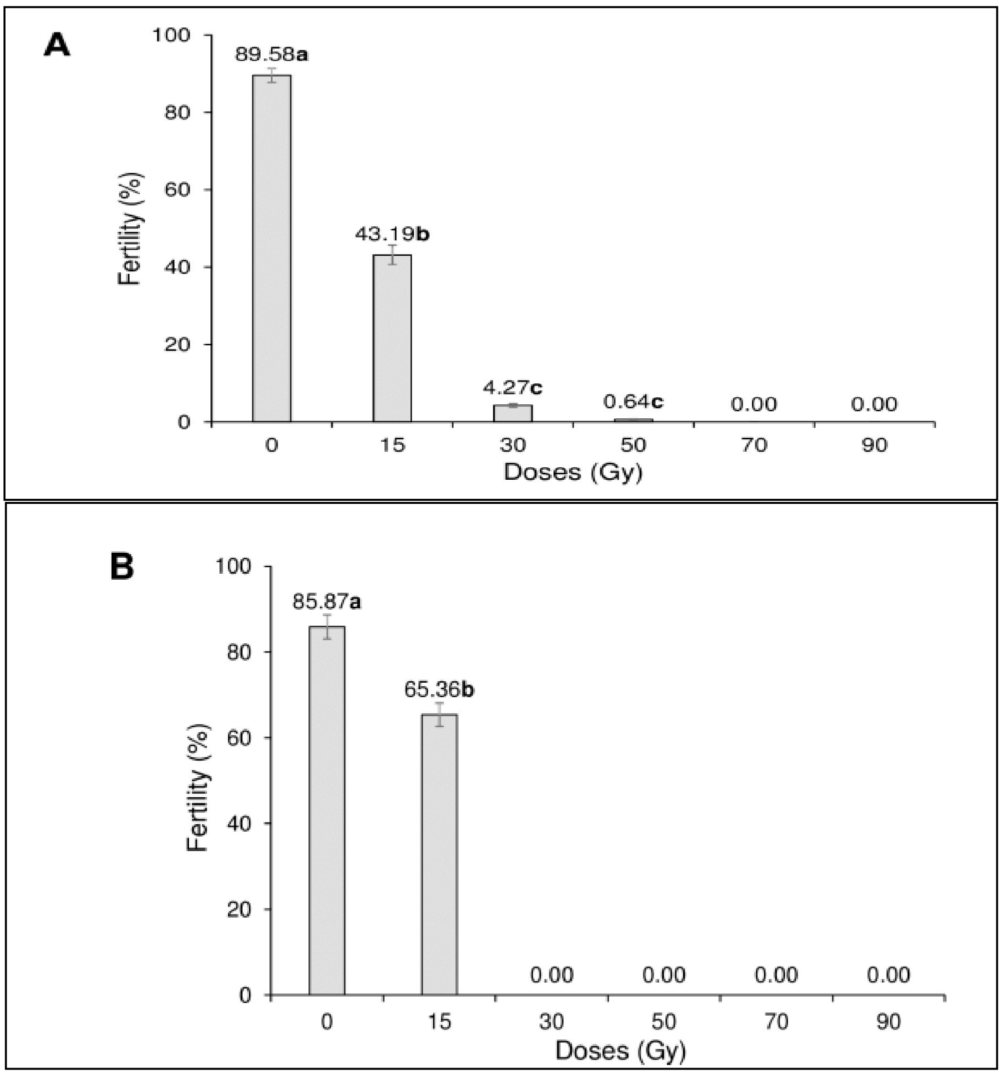


Figure 9: Effects of irradiation on percentage of egg fertility of *Aedes aegypti*. (A) Irradiated males mated with non-irradiated females and (B) irradiated females mated with non-irradiated males **(Bond et al., 2019)**

The Fertility decreased due to damage to ovarian tissue, which led to the inability to produce eggs. Since egg production decreased at 30 Gy for females irradiated, and fertility in males irradiated decreased at the rate of irradiation 70 and 90 Gy this seemed to indicate that females were more affected by radiation.

The fourth study (the sixth article) **(Gato et al., 2021)**.

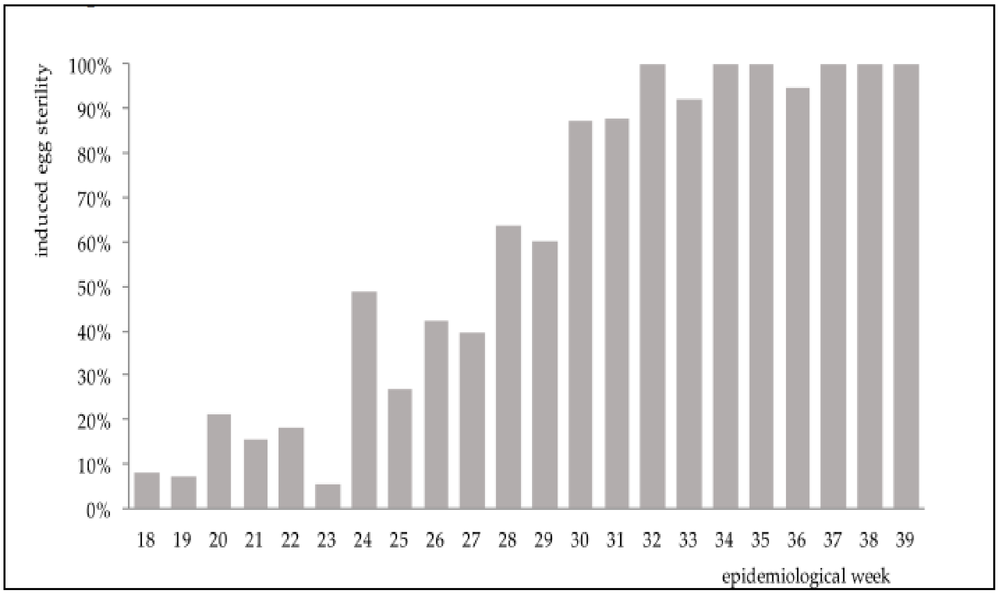


Figure 10: Weekly mean induced egg sterility (%) in the release site El Cano between epidemiological weeks 18 and 39, 2020**(Gato et al., 2021)**.

Chossing a release site is important to obtain reliable data and ensure the success of this technique, as the *Ae.aegypti* is an urban mosquito and was released in urban areas and also ovitraps represents an effective way to monitor mosquito population . The number of absolute sterile males compared to wild males was low due to the inability to mass breed .

As for *Ae.aegypti*mosquito they found in the first article. **(Ernawan et al., 2022)** that exposing pupae or adults to 100 Gy caused them complete sterility , and exposing them to 70Gy caused them to be sterile 98%, that is what confirmed in other articles. As for the second article **(Ernawan et al., 2018)** , when irradiating adult males with 70Gy after mating them , the rate of sterility did not decrease below . In the third article **(Bond et al., 2019)** , the rate of infertility was completely eliminated at . As for the fourth article **(Gato et al., 2021),** they discussed of the releases site and the release site and the number of release. From these actions, we conclude that the best dose was 70Gy, as it caused infertility ranging from 98% to 100% and it did not occur at, this dose has significant effects on competitive ability.

- ***Anopheles stephensi***

There is only one study that talks about *Anopheles stephensi* (seventh article **(Yadav et al., 2010)**). After each blood meal, females that mated with males irradiated with 100 Gy exhibited reduced fertility compared to those mating with males irradiated with 70 Gy. Additionally, pupae irradiated with 100 Gy showed a sterilization rate of 98.6%, whereas those irradiated with 70 Gy had a sterilization rate of 83.4%. Reduced fertility in mosquitoes results in fewer eggs laid, ultimately leading to a decline in mosquito populations and potentially limiting the spread of disease vectors. Irradiation aims to effect germ cells in organism, but is not specific, causing physical harm, including reducing longevity.

2 Precision guided sterile insect technique pgSIT

- ***Aedes aegypti***

In both articles 1 **(Li et al., 2021)** they found that both *myosin heavy chain* gene are necessary for flight and *β-Tubulin* 85D gene are necessary for fertility , and that when they are disabled, fertility decreases and the mosquito becomes unable to fly. As for article 2 **(Li et al., 2024)**, they found that when both *ix* or *dsx* genes were disabled, this led to morphological deformities, and among these deformities were distorted ovaries and abnormal reproductive organs , which in turn to a small number of eggs that could not be hatched.

In the article 1 **(Li et al., 2021)**,to ensure the effectiveness of the pgSIT technology, egg release was simulated, in which 400 pgSIT egg was released for every *Aedes aegypti* adult for a period of 10 _ 24 week. With taking into account both the number of mosquitoes that the female lays daily in a temperate climate, which is estimated at more than 30 eggs and the decrease in the competitive ability of the males by a percentages 25% although this has not been shown in laboratory experiments. So the rate of population elimination reached more than 96%.

The role of pgSIT technology in disabling essential genes throughout the development period led to the production of mosquitoes females that are unable to fly, and sterile male, and has a short lifespan. The mosquitoes resulting from this technology are considered to have the ability to compete with WT.

In the same way as was done in article 2 **(Li et al., 2024)** but with a different number of pgSIT eggs, a field simulation was conducted in which 250 pgSIT eggs was released (a release scheme of ~26 releases) for each *Aedes aegypti* adult mosquito and the results showed that fertility and the rate life did not exceed ~0.01.

In article 2**(Li et al., 2024)** , it was mentioned that one of the reasons why fertility did not end 100% is that they used a small number of gRNAs targeting *βTub* genes . They only used 2gRNAs.

3 Chemosterilants

We chose three types of chemosterilants thiotepa, apholate and amide to work on.

- ***Culex pipiens***

Hafez and Aboul-Nasr and Salama obtained the following results in the year 1971 for applying thiotepa and apholate

Table 6: Chemosterilization of *Cx. pipiens* by larval treatments**.**

Concn (ppm)	No. egg rafts/ female after 2 blood meals	Mean no.egg/raft	Hatching larvae /raft	% hatching
Apholate				
100	2	85	61	71.7
200	2	65	42	64.6
300	2	71	35	49.3
400	2	49	0	0
Control	2	92	64	69.5
Thiotepa				
5	2	70	49	70.0
10	All larvae died			
15	All larvae died			

20	All larvae died			
Control	2	84	62	73.8

Table 7: Chemosterilization of *Cx.pipiens* by adult food treatment.

Conch of chemo-sterilant in sugar (%)	Treated sex	No.egg rafts after 2 blood meals	Mean no.egg/raft	Hatching larvae/raft	% Hatching
Aopholate					
0.01	Both	2	70	29	41.4
	Female	2	64	40	62.3
	Male	2	83	50	60.2
0.1	Both	0	0	0	0
	Fomale	0	0	0	0
	Male	2	49	0	0
1	Both	0	0	0	0
	Female	0	0	0	0
	Male	2	45	0	0
Control		2	98	76	77.5
Thiotepa					
0.001	Both	2	50	30	60.0
	Female	2	80	54	67.5
	Male	2	72	47	65.2
0.01	Both	2	60	25	41.6
	Female	2	54	22	40.7
	Male	2	68	5	51.4
0.05	Both	0	0	0	0
	Female	0	0	0	0
	Male	1	65	0	0

Control		2	78	55	70.5

The results indicate that complete sterility in *Cx. pipiens* larvae was achieved at a concentration of 400 ppm of Apholate. Thiotepa, on the other hand, did not induce sterility at lower concentrations (5 ppm) but was toxic at higher concentrations (10-20 ppm), in terms of adult food treatments, Apholate at 0.1% concentration resulted in complete sterility in *Cx. pipiens* when both sexes or separate sexes were treated, Thiotepa was more effective, achieving complete sterility at a lower concentration of 0.05% for both sexes of *Cx. pipiens*.

In conclusion, it can be said that Thiotepa is considered more effective than Apholate in sterilizing Cx. *pipiens*.

- ***Aedes aegypti***

In the year 2014, Gato obtained the following results of the effect of Thiotepa in sterilizing *Aedes aegypti*

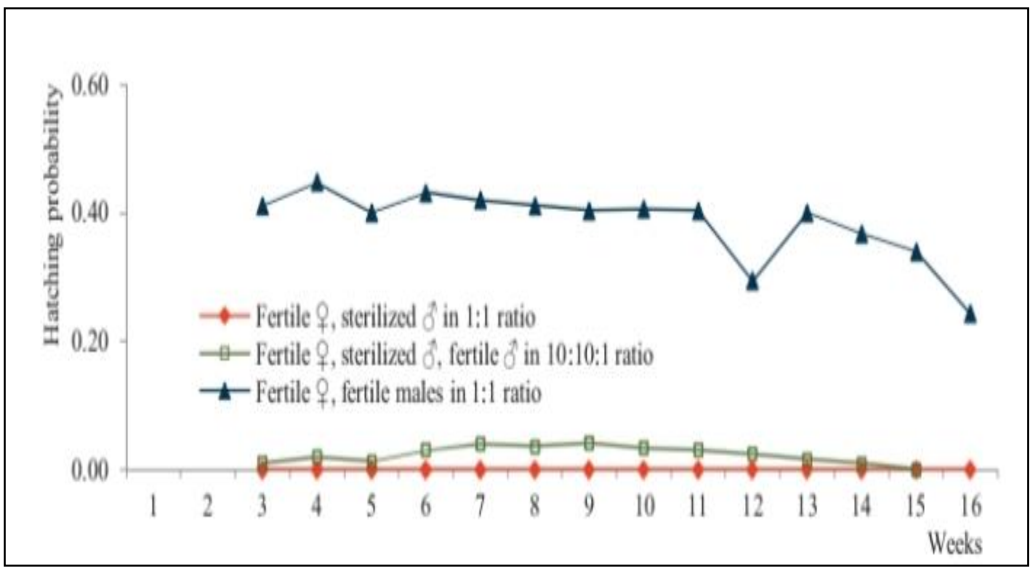


Figure 11: Hatching probability (Hx) of *Ae. aegypti* in the population cage experiment.

Table 8 : Life-table parameters of *Ae.aegypti* in the population cage experiments.

Ae. aegypti cage	Net reproduction rate (R0)	Mean generation time	Finite rate of natural	Intrinsic rate of natural

population		(T)	increase	increase (r)
Fertile ♀, sterilized ♂ in 1:1 ratio	0a	0a	0a	0a
Fertile ♀, sterilized ♂, fertile ♂ in 10:10:1 ratio	15.8b	7.3b	1.57b	0.45b
Fertile ♀, fertile males in 1:1 ratio	141.2c	7.1b	2.92c	1.07c

Letters following the numerical values denote the results of multiple comparison tests; similar letters in the same column indicate no significant difference.

The study focused on the release of Thiotepa-sterilized males into caged populations of *Aedes aegypti* mosquitoes and analyzed the life table parameters to assess the impact on reproductive potential., Thiotepa, a chemosterilant, was used at a concentration of 0.6% in distilled water buffered to pH 8.6, the results showed that the presence of Thiotepa-sterilized males did not affect the survival of the mosquitoes but significantly reduced the fecundity of females, as indicated by hatch rate and stable age structure parameters, the life table analysis revealed that populations with thiotepa-treated males had lower net reproduction rates, finite rates of natural increase, and intrinsic rates of natural increase, suggesting that these populations would not be able to proliferate in natural conditions, this indicates that the release of thiotepa-treated males could effectively reduce the reproductive capability of the target mosquito population and contribute to vector control efforts.

According to Rai, KS in the year 1964, we obtained the following results for the effect of Apholate on *Aedes aegypti*:

Table 9: Effect of 15 pf>m Apholate on the fertility and fecundity of *Aedes aegypti*.

Cross*	1st blood meal		2nd blood meal		3rd blood meal	
Female Male	Eggs laid / ♀	%Eggs hatched / ♀	Eggs laid /♀	%Eggs hatched / ♀	Eggs laid /♀	%Eggs hatched / ♀

U × U	109 (12) **	97.7	113 (11)	97.6	115 (9)	96.7
U × T	100 (18)	10.0	109 (14)	5.7	86.5 (13)	4.4
T × U	7 (11)	34.0	4 (9)	68.2 ***	5 (9)	29.6
T × T	3 (17)	5.6	2 (15)	18.0	1 (12)	0.0

*** U = untreated; T = treated; ** Figures in parentheses represent number of single pair crosses made; *** Only one 9 laid 39 eggs, out of which 27 hatched.**

Treating female larvae with 15 ppm Apholate resulted in almost complete infecundity, with most treated females either not ovipositing or laying very few eggs, this significant impact on fecundity highlights the potent sterilizing effect of Apholate. The inhibition of ovarian development in treated females, characterized by impaired growth and differentiation of follicles leading to degeneration, further supports the sterilizing mechanism of Apholate, Additionally, the observation of induced dominant lethal in the sperm of treated males explains the reduced fecundity and fertility in males, the necrosis of the testicular epithelium in treated males further emphasizes the disruptive effects of Apholate on reproductive tissues, the concentration of 15 ppm and the duration of exposure from larval stages until pupation were crucial in achieving the sterilizing effects observed in the study, these results underscore the effectiveness of Apholate as a chemosterilant in disrupting the reproductive capabilities of *Aedes aegypti*.

Thiotepa appears to be more effective than Apholate in sterilizing *Aedes aegypti* mosquitoes, the study demonstrated that the release of Thiotepa-sterilized males significantly reduced the fecundity of female mosquitoes and impacted their reproductive potential, leading to lower net reproduction rates, finite rates of natural increase, and intrinsic rates of natural increase, this suggests that Thiotepa-treated males could effectively reduce the reproductive capability of the target mosquito population, we also note that hatching probability was zero when using Thiotepa and the number eggs hatched when using Apholate became zero only at the third meal of blood, However, without specific data on the effectiveness of Apholate in a similar context, it's challenging to make a direct comparison between the two chemosterilants.

- ***Anopheles stephensi***

During a study conducted by Sharma and Razdan and Ansari (1978) on the effect of chemosterilant on sterilization *Anopheles stephensi* the following results were obtained:

Table 10 : Effect of 1 % Thiotepa solution on fertility and fecundity of *An. stephensi*.

Expo-sur (min)	**Sterility in males**		**Sterility in females**	
	Total eggs obtained	Sterility (%)	Total eggs obtained	Sterility (%)
30	21546	39.1	13057	24.3
60	10045	60.6	5140	50.7
90	4895	83.5	2846	64.2
120	9007	85.7	3289	54.7
150	14646	92.8	4095	67.4
180	9932	84.0	9637	41.3
210	9822	88.5	6894	32.4
0	4746	1.2		

Table 11: Effect of 1% P, P-bis (I-aziridinyl)-N-methyl·phosphinothioic amide on fertility and fecundity of *An. stephensi*

Sex treated	Expo-sure (min)	Total eggs obtained	Sterility (%)	Eggs obtained per female
Male	30	4333	91.6	43.3
	60	1131	91.3	31.3
	90	4305	98.4	43.1
	120	5671	100.0	56.7
	150	3179	100.0	31.8
Female	30	2257	71.7	22.8
	60	984	94.9	9.8
	90	138	99.3	1.4
	120	62	100.0	0.6
	150	0	100.0	0.0
	0	4134	1.7	41.3

Thiotepa was found to be ineffective in inducing 99-100% sterility in both males and females, even with prolonged exposure times, on the other hand, the Amide was highly successful in achieving 100% sterility in both sexes.

Specifically, mentions that a 120-minute exposure to a 1% solution of "the amide" resulted in 100% sterility in males, this concentration and exposure time were crucial in achieving maximum sterilization effectiveness in male *Anopheles stephensi* mosquitoes, the fact that "the amide" could achieve complete sterility in both males and females highlights its potency as a chemosterilant for controlling mosquito populations.

The results also indicate that the mating competitiveness of chemosterilized males was higher than expected, suggesting that the sterilization process did not compromise their ability to compete for mates.

Furthermore, the permanency of sterility in chemosterilized males, showing that the sterility induced by Amide remained effective even after aging the males for different periods, this long-lasting effect is essential for ensuring sustained population control of *Anopheles stephensi* mosquitoes.

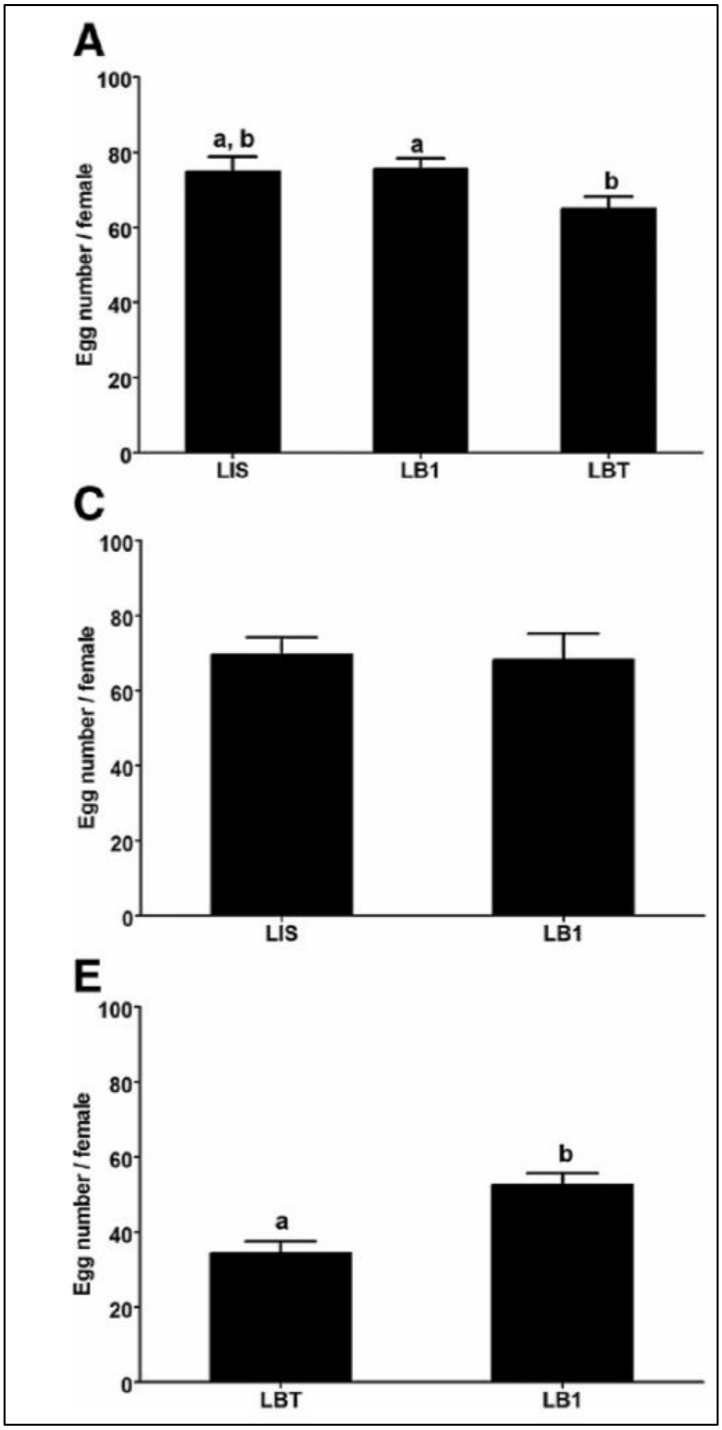
In conclusion, the results highlight the importance of selecting the right chemosterilant and optimizing the concentration and exposure time to achieve maximum effectiveness in sterilizing *Anopheles stephensi* mosquitoes, "The amide" emerges as a promising chemosterilant that can induce 100% sterility in both males and females, making it a valuable tool in mosquito population control efforts.

4 Wolbachia induced cytoplasmic incompatibility

- ***Anopheles stephensi***

In the year 2014 Joshi studied the effectiveness of *Wolbachia* bacteria on sterilizing mosquitoes *Anopheles stephensi*:

The presence of wAlbB was found to reduce the fecundity of female mosquitoes, impacting the number of eggs laid and the hatch rate of those eggs compared to wild-type mosquitoes. Additionally, wAlbB had a minor effect on the mating competitiveness of male mosquitoes, interestingly, wAlbB increased the lifespan of both male and female mosquitoes when they were solely maintained on sugar meals, but did not impact the lifespan of blood-fed females, this suggests a complex interaction between *Wolbachia* infection and feeding behavior in mosquitoes. furthermore, the study highlighted that the concentration and duration of exposure to *Wolbachia* were crucial factors in achieving desired outcomes, specifically, to achieve sterility in *Anopheles stephensi* mosquitoes, it is essential to carefully control the concentration of *Wolbachia* and the duration of exposure to ensure effective sterilization without compromising other aspects of mosquito fitness.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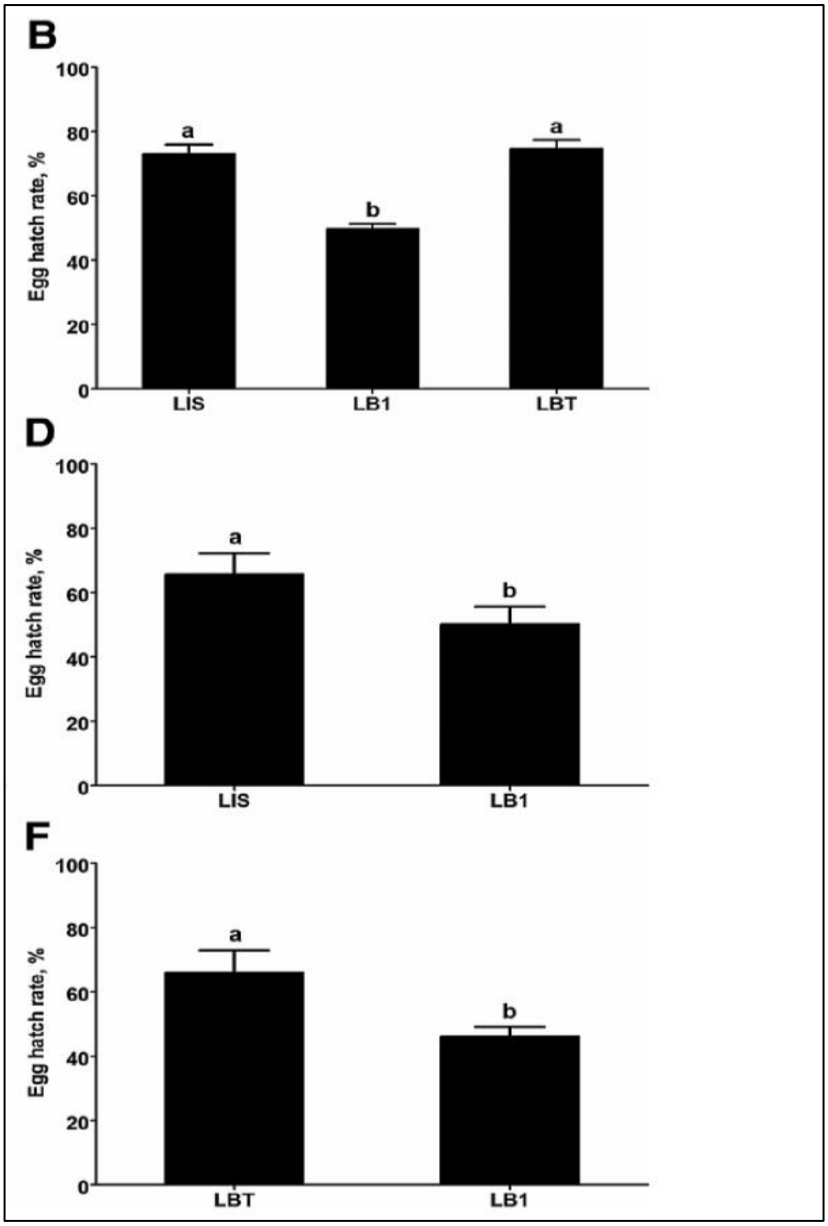


Figure 12: Impact of wAlbB on *An. stephensi* fecundity.

The number of eggs laid by each individual female and the hatch rate after feeding onmouse (A, B), sheep (C, D) or human (E, F) blood, for all figures, error bars represent standard error; statistical significance is represented by letters above each column, with different letters signifying distinct statistical groups [$P < 0.05$; student's t-test for (A and E); $P < 0.0001$; chi-squared test for (B, D and F)].

- ***Culex pipiens***

In the year 2011 atyame studied the effectiveness of sterilization *Culex pipiens* using *Wolbachia* bacteria and the results were as follows:

Table 12: Reciprocal crosses between LR [wPip (Is)] line and field specimens.

Crosses	Hatching rate	Outcomes
♂ LR [wPip(Is)] × ♀ Samuel	0.011± 0.006 (≥9000; 75) a	bidirectional CI
♂ Samuel × ♀LR [wPip(Is)	0.000±0.000 (≥ 4400; 36) a	
♂ LR [wPip(Is)] × ♀Salines	0.007± 0.005 (≥ 6000; 51) a	bidirectional CI
♂ Salines × ♀ LR [wPip(Is)]	0.000± 0.000 (≥4500; 37) a	
♂ LR [wPip(Is)]× ♀ Grande Glorieuse	0.000±0.000 (≥12000; 97) a	bidirectional CI
♂ Grande Glorieuse × ♀ LR [wPip(Is)]	0.000±0.000 (≥ 500; 40) a	
♂LR [wPip (Is)] ×♀Tsoundzou	0.000± 0.000 (≥9000; 75) a	uni- and bidirectional CI
♂ Tsoundzou ×♀ LR [wPip(Is)]	0.112±0.092 (≥2000; 16) b	
♂ LR [wPip(Is)]× ♀ Mada	0.000±0.000 (≥5000; 44)a	uni- and bidirectional Cl
♂ Mada ×♀ LR [wPip(Is)]	0.804±0.283 (≥2500; 20) c	

Field specimens are from Samuel (La Re´union), Salines (Mauritius), Grande Glorieuse, Tsoundzou (Mayotte) and Mada (Madagascar), for each cross, mean hatching rate.

± standard error, number of eggs and egg-rafts are reported.

a, b and c represent statistical groups (Wilcoxon two sided-test with Bonferonni's adjustment for multiple comparisons), note that, in the cross ♂ Tsoundzou ×♀

LR [wPip(Is)], 14 males induced complete CI while 2 were compatible; in the cross ♂ Mada ×♀ LR[wPip(Is)], 2 males induced complete CI and 18 were compatible

(see text for more details).

The study introduces the LR [wPip(Is)] line, which was created by introducing the *Wolbachia* wPip(Is) strain into *Cx. p. quinquefasciatus* mosquitoes from La Reunion. The results showed that LR [wPip (Is)] males induced total embryonic lethality when crossed with field females from the islands, indicating a high level of sterilization. Additionally, LR [wPip (Is)] males were found to be equally competitive with wild males in mating, suggesting their effectiveness in reducing the population, the also highlighted the bidirectional incompatibility between LR [wPip (Is)] females and field males, reducing the risk of *Wolbachia* replacement in the wild population, the demonstrated the feasibility of an Incompatible Insect Technique (IIT) using LR [wPip (Is)] males to control *Cx. p. quinquefasciatus* populations in the region.

While in the investigation conducted by Rasgon and Scott in 2003 concerning *Wolbachia* infection within California *Culex pipiens* populations, notable findings were observed, particularly the achievement of complete sterilization in incompatible crosses, both within laboratory and field settings, this outcome presents a promising avenue for leveraging *Wolbachia* as a prospective tool within vector control strategies, the auteur delineates the sterilization process, outlining the rearing of newly hatched larvae in a tetracycline solution at varying concentrations (50 ppm for two generations, 100 ppm for four generations, and 200 ppm for two generations) to eradicate *Wolbachia* infection. Additionally, the provision of food at the onset of rearing was instrumental in mitigating larval mortality during treatment, following the third generation of tetracycline treatment, *Wolbachia* infection became undetectable, indicative of successful sterilization of *Culex pipiens*, this meticulous protocol underscores the efficacy of *Wolbachia* induced sterilization within mosquito populations.

Table 13: hatch rate in all four possible crosses between LIN (infected) and LINT (uninfected) *Cx. pipiens*.

Cross	N	Mean	95% C.I.
LIN×LIN	16	0.657	0.492-0.819
LIN×LINT	35	0.801	0.657-0.883
LINT × LIN[a]	33	0	_
LINT×LINT	64	0.852	0.779-0.896

Crosses were female × male.

[a] Mean is significantly different (P≤ 0.0001) from all other

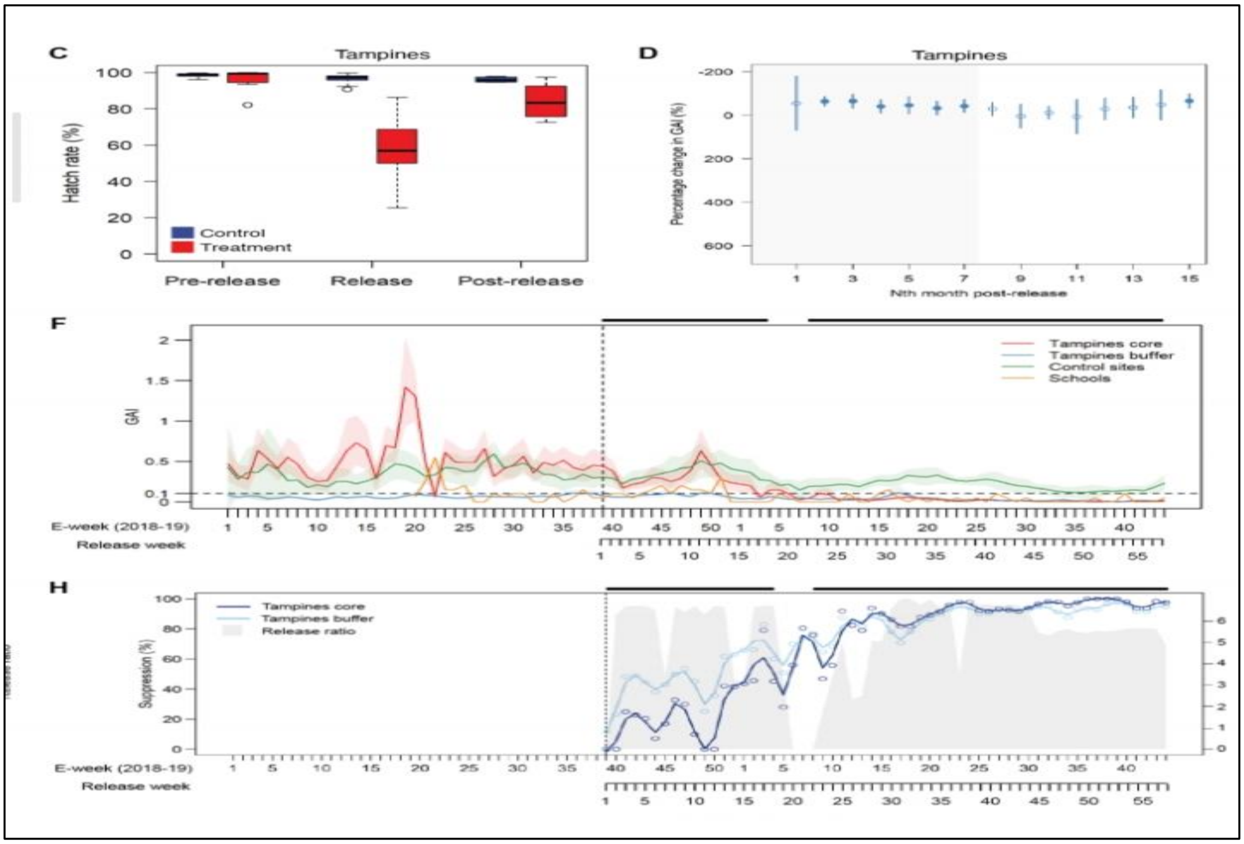
means after correcting for multiple comparisons. Confidence intervals were calculated by bootstrapping (5000 replicates).

The reason for the difference in results between Atyame and Rasgon, Scott is due to several reasons, most notably the difference in geographical region and the different *Wolbachia* strains used in each study, in addition to not examining the effect of male age on cytoplasmic incompatibility in the study conducted by Rasgon, Scott In contrast to what he did atyame therefore, we will necessarily obtain different results, as well as differences in the experimental and methodological settings for each work.

- ***Aedes aegypti***

The results from a study in 2021 on the use of *Wolbachia* infected *Aedes aegypti* mosquitoes to suppress populations and reduce dengue incidence in high-rise urban areas in Singapore present that releases of male *Wolbachia* infected mosquitoes led to a significant reduction in wild type *Aedes aegypti* populations and a corresponding decrease in dengue incidence,the utilized two strategies: IIT-SIT in Yishun and high-fidelity sex-sorting in Tampines, both resulting in profound suppression of mosquito populations. In Yishun, irradiation at 30 Gray was used to sterilize residual females, achieving 100% female sterility, in Tampines, a high-fidelity sex-sorting pipeline reduced female contamination rates significantly, the results showed that the suppression of mosquito populations was sustained, with the establishment of

Wolbachia being prevented through these methods, also highlighted was the importance of maintaining low wild type mosquito populations to prevent establishment and the need for additional measures, such as SIT, to mitigate establishment if it occurs.



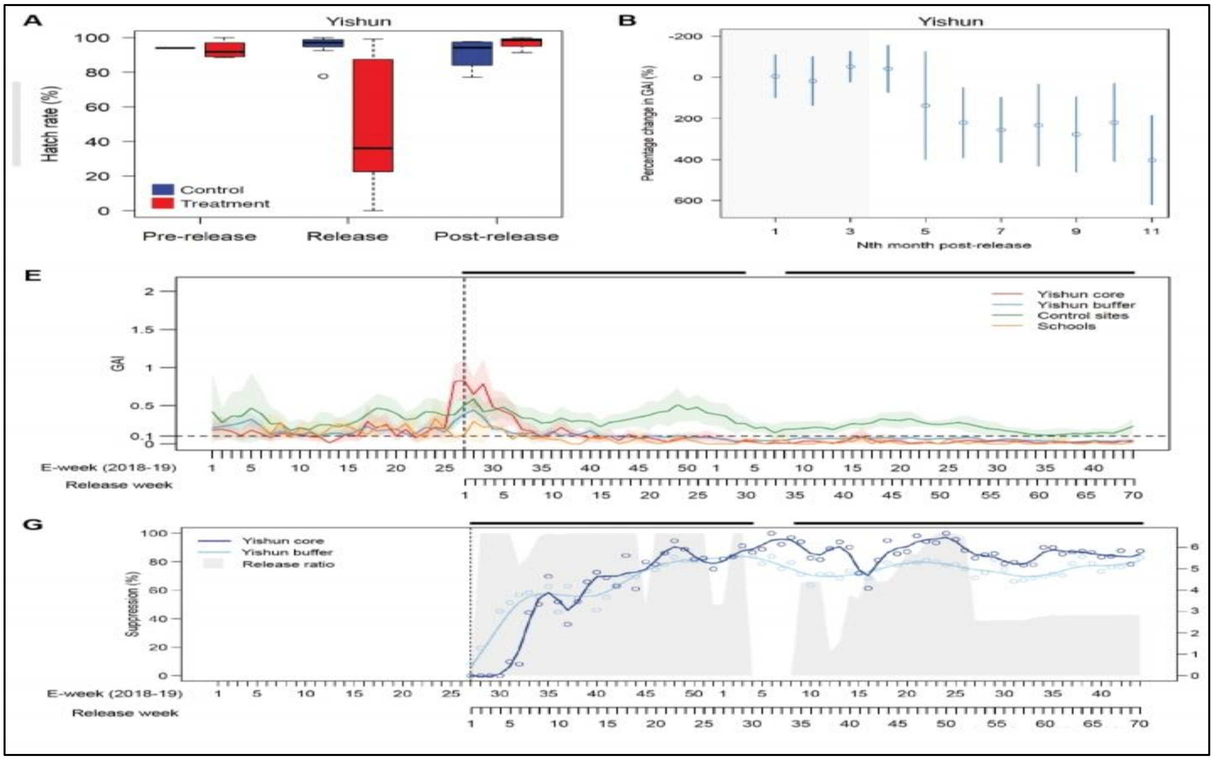


Figure 13: Suppression of *Aedes aegypti* mosquito populations following Phase 1 (A-D) and Phase 2 (E-H) releases of wAlbB-SG males.

(A) Weekly egg hatch rates in the Yishun treatment and control sites pre-, during, and post-Phase 1releases. Lines and limits of the boxes indicate medians and 25&' and 75&' percentiles respectively; whiskers extend to 1.5 times the interquartile range. Outliers are shown as points. (B) Monthly BACI suppression estimates of GAI in the Yishun treatment site during (grey-shaded region) and post-Phase 1release. Point estimates and 95% confidence intervals are shown; negative values indicate suppression. Filled circles indicate statistical significance ($p<0.05$). (C-D) Same as (A-B) but for the Tampines treatment and control sites. (E-F) Weekly GAI in control sites and in core release zones, buffer release zones, and schools in the Yishun (E) and Tampines (F) Phase 2 study sites. Schools did not receive releases. Shaded areas indicate 95% CIs. The horizontal dotted line indicates the lowdengue risk GAI threshold of 0.1. (G-H) Weekly adult female *Aedes aegypti* suppression compared to control sites and weekly release ratios (number of wAlbB-SG males released per resident per week) in the Yishun (G) and Tampines (H) Phase 2 study sites. The start of Phase 2 releases is indicated by the vertical dotted line, and release periods are denoted by horizontal black bars. Release sites were expanded following the pause in releases.

In 2023 Lim found the following results; Intention-to-treat analyses showed that releasing *Wolbachia* infected *Aedes aegypti* males for 12 months or more was associated with a significant protective efficacy ranging from 36% to 77%, depending on the township, and from 48% to 78% across different years, notably, the study demonstrated that longer durations of *Wolbachia* exposure were linked to greater risk reductions of dengue, the findings suggest that repeated releases of *Wolbachia* infected males can effectively suppress wild type mosquito populations and reduce disease transmission, offering a potential strategy for strengthening dengue control in tropical cities where the burden of the disease is high.

The variation in outcomes between the two studies can be attributed to the adoption of advanced strategies, such as IIT-SIT and high-fidelity sex sorting, in the 2021 study conducted by the Singapore Consortium, innovative techniques were also employed, with a focus on maintaining low wild type mosquito populations and implementing additional measures to prevent *Wolbachia* establishment, furthermore, differences in surrounding conditions and environmental factors also contributed to the disparate results.

Conclusion

Conclusion

This study was conducted in order to find out the most effective methods for sterilizing mosquitoes due to their importance in spreading diseases, and given the huge number of existing mosquito species , we chose three of them because they are widespread and they *Aedes aegypti* , *Culex pipiens* , *Anopheles stephensi* through our research , we found that there are 4 methods of sterilization the first of which was SIT using radiation , specifically gamma rays , due to their great spread , as the rate of sterilization of a *Cx. pipiens* mosquito was 96,1% when using a dose of radiation estimated at 80Gy , as for *Ae.Aegypti*, the best irradiation dose was considered to be 70Gy and the rate of sterilization when used reached 98%, as for *Anopheles stephensi*, when it was irradiated with 100Gy the sterility rate was 98, 6%. As for the second technology, it was a pgSIT technology and all the work available for it related to the *Ae. aegypti* only and they found that it caused the elimination of the population of the same mosquito by a percentage of 96% when the gene responsible for fertility *β-Tubulin* 85D and the gene responsible for flight *myosin heavy chain* were disabled. As for the technology number three, it was Chemosterilant . They found that when a mosquito *Cx. Pipiens* achieved Thiotepa complete sterility at a concentration estimated at 0.05% and when it was used on *Ae. aegypti* at a concentration 0.6% it reduced female fertility significantly, as for the *An.Stephensi* exposing it to 1% from amide caused her complete sterility. As for the last technology, it was Wolbachia induced CI which is a type of bacteria and it had an effect on the fertility of *Anopheles stephensi* female , as for the *Cx. pipiens* it caused her complete sterility and finally it led to a decrease in the population *Ae.aegypti* mosquito of the same specie infected with it were released.

We will not be able to deny the effectiveness of any one of these technique in sterilizing mosquitoes, but because the technique that relies on rays has a negative impact on the competitive ability of males, and because of the negative effects of both the technic Chemosterilant and Wolbachia induced CI on the environment, it remains a pgSIT it is the best.

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86.

Summury

Résumé

Le moustique transmet de nombreuses maladies dangereuses aux humains et aux animaux, en raison de ce danger significatif, les chercheurs ont développé des méthodes pour le combattre, notamment des techniques de stérilisation, sur lesquelles notre travail se concentre, nous avons sélectionné quatre types de méthodes de stérilisation bien connues et hautement efficaces : le Chemosterilant, l'incompatibilité induite par *Wolbachia*, l'irradiation et le pgSIT, nous avons évalué l'efficacité de l'application de ces méthodes sur trois des espèces de moustiques les plus prévalentes : *Ae. aegypti*, *An. stephensi* et *Cx. pipiens*, l'objectif était de déterminer la meilleure technique de stérilisation parmi les quatre, en conclusion, nous avons constaté que l'irradiation a un impact négatif sur la capacité concurrentielle des mâles, tandis que le Chemosterilant et l'incompatibilité induite par *Wolbachia* ont des effets négatifs sur l'environnement, cependant, le pgSIT était exempt de ces inconvénients et s'est distingué par un taux de stérilisation de 96% pour la population de moustiques, par conséquent, le pgSIT est considéré comme le meilleur parmi les quatre techniques.

Mots Clés: Moustique, Stérilisation, Chimiostérilant, Irradiation, pgSIT.

Summary

The mosquito transmits many dangerous diseases to humans and animals, due to this significant danger, researchers have developed methods to combat it, including sterilization techniques, which our work focuses on, we selected four types of well-known and highly effective sterilization methods: Chemosterilant, *Wolbachia* induced incompatibility, Irradiation, and pgSIT, we assessed the effectiveness of applying these methods on three of the most prevalent mosquito species: *Ae. aegypti*, *An. stephensi*, and *Cx. pipiens*, the aim was to determine the best sterilization technique among the four, in conclusion, we found that Irradiation has a negative impact on the competitive ability of males, while Chemosterilant and *Wolbachia* induced incompatibility have negative effects on the environment, however, pgSIT was free from these drawbacks and distinguished itself with a sterilization rate of 96% for the mosquito population, therefore, pgSIT is considered the best among the four techniques.

Keywords: Mosquito, Sterilization, Chemosterilant, Irradiation, pgSIT.

ملخص

ينقل البعوض العديد من الأمراض الخطيرة على الإنسان و الحيوان ونظرا لهذه الخطورة الكبيرة للبعوض قام الباحثون بتطوير طرق لمكافحتها من ضمنها طرق التعقيم التي ينصب عليها عملنا حيث قمنا بالاختيار أربع أنواع من طرق التعقيم المشهورة و الأكثر فعالية و هي Chemosterilant و Wolbachia induced incompatibility و Irradiation و pgSITو قسنا مدى فعالية تطبيقها على ثلاث أنواع من البعوض الأكثر خطوة و التي طبقت عليها هذه التقنيات بكثرة وهي *Ae. aegypti* و*An. stephensi* و*Cx. pipiens* بهدف تحديد أفضل تقنية لتعقيم من بين التقنيات الأربعة وفي الختام وجدنا أن تقنية Irradiation لها تأثير سلبي على القدرة التنافسية لذكور وتقنية Chemosterilant و Wolbachia induced incompatibility لديها تأثيرات سلبية على البيئة بينما تقنية pgSIT فكانت خالية من هذه العيوب و تميزت بنسبة تعقيم تقدر 96% لمجمع البعوض وبالتالي يعتبر pgSIT هو الأفضل بين التقنيات الأربعة.

الكلمات المفتاحية : البعوض, التعقيم, pgSIT, Irradiation, Chimiostérilant.