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Effects of diet on the longevity and insecticide resistance of *Anopheles gambiae*

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Abstract

In addition to feeding on blood, mosquitoes commonly consume nectar, which provides essential nutrients like sugars as well as plant-derived compounds known as phytochemicals. Nectar serves as their primary energy source and supports key life-history traits such as longevity. While the genetic mechanisms underlying insecticide resistance are well documented, the influence of environmental factors such as diet remains poorly understood. Despite the well-established effects of several phytochemical constituents of nectar on living organisms, their role in modulating mosquito longevity and insecticide susceptibility has been little explored.

To address these gaps, we aimed to (i) assess the effect of diet on mosquito longevity, (ii) explore the impact of nectar and certain phytochemical constituents of nectar on the insecticide resistance phenotype, (iii) examine how diet influences both resistance and longevity in mosquitoes infected with *Plasmodium falciparum*.

Overall our results show that the longevity of mosquitoes varies from one plant species to another, although the concentration of total sugar alone in the nectar does not significantly explain this longevity. Additionally, the plant-based diets had no effect on mosquito resistance to insecticides. In contrast, the tested phytochemical compounds significantly influenced mosquito responses to insecticide. The tested alkaloid, as part of the diet, affected the insecticide response of infected mosquitoes but did not influence the longevity of survivors following exposure.

Overall, these findings highlight the importance of nectar and sugar feeding in determining the longevity of mosquitoes, but suggest that non-sugar components of nectar have a large impact. Also, they underline the complex role of the mosquitoes' diet on their response to insecticides.

Keywords: resistance, insecticide, longevity, mosquito, nectar, diet, blood meal, phytochemicals, oxidant, antioxidant, *Plasmodium falciaparum.*

Résumé

En plus du sang, les moustiques consomment régulièrement du nectar, qui leur fournit des nutriments essentiels, principalement des sucres, mais aussi divers métabolites primaires et secondaires. Le nectar constitue leur principale source d'énergie et soutient des traits clés de leur biologie, tels que la longévité. Si les mécanismes génétiques responsables de la résistance aux insecticides sont bien documentés, l'influence des facteurs environnementaux, tels que l'alimentation, demeure mal comprise. Malgré les effets bien établis de plusieurs constituants phytochimiques du nectar sur les organismes vivants, leur rôle dans la modulation de la longévité et la sensibilité des moustiques aux insecticides a été peu exploré.

Afin de combler ces lacunes, nous avons cherché à (i) évaluer l'effet de l'alimentation sur la longévité des moustiques, (ii) explorer l'impact du nectar et de certains composés phytochimiques du nectar sur le phénotype de résistance à l'insecticide, et (iii) examiner comment l'alimentation influence à la fois la résistance à l'insecticide et la longévité chez les moustiques infectés par *Plasmodium falciparum*.

Dans l'ensemble, nos résultats montrent que la longévité des moustiques varie selon les espèces végétales, bien que la concentration totale en sucres dans le nectar à elle seule n'explique pas significativement cette variation. De plus, les régimes alimentaires à base de plantes n'ont pas affecté la résistance des moustiques aux insecticides. En revanche, les composés phytochimiques testés ont exercé une influence significative sur la réponse des moustiques aux insecticides. Le régime alimentaire à base d'alcaloïde a affecté la réponse des moustiques infectés à l'insecticide, mais n'a pas influencé la longévité des survivants après exposition.

Globalement, ces résultats soulignent l'importance du nectar et de la consommation de sucres dans la détermination de la longévité des moustiques, mais suggèrent que les composants non sucrés du nectar ont un impact important. Ils mettent également en évidence le rôle complexe de l'alimentation des moustiques sur leur réponse aux insecticides.

Mots-clés : résistance, insecticides, moustique, longévité, nectar, régime alimentaire, repas sanguin, composés phytochimiques, oxydant, antioxydant, *Plasmodium falciparum*.

Contents

Chapter 1: General introduction

1.1. Background

Malaria: burden and overview

The term “malaria” from the Italian mal'aria (meaning "bad air"), reflects the disease's historical link to marshy areas [1]. Though known since ancient Egypt, malaria was only linked to the *Plasmodium* parasite in 1880 by Charles Louis Alphonse Laveran [2]. In 1897, Ronald Ross discovered its transmission cycle via mosquito bites, and in 1898, Giovanni Battista Grassi confirmed *Anopheles* mosquitoes as the vectors, marking a key milestone in malaria research [3].

Malaria remains the leading cause of death from vector-borne diseases [4]. In 2021, it caused 593,000 deaths in sub-Saharan Africa alone [4]. Endemic to tropical and subtropical regions, [5] malaria is transmitted to humans through the bites of infected female *Anopheles* mosquitoes [6]. Five *Plasmodium* species infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* [7]. In Africa, *Anopheles gambiae* s.l. is a major vector, with *P. falciparum* causing the most severe cases [8] [6] [9]. The highest risk groups are young children without acquired immunity and pregnant women with reduced immunity [10]. In sub-Saharan Africa, a child under five dies from malaria every 1 to 2 minutes [4].

Malaria, often referred to as “the epidemic of the poor”, particularly affects the most deprived populations, who do not have access to the means of prevention and treatment [11]. It constitutes a heavy economic burden, slowing down growth, perpetuating poverty and hindering the development of the countries affected [11]. Direct costs, which include medical (treatment and control) and non-medical (transport, accommodation, and food) [12] and indirect costs, such as absenteeism from work and the effects on children's education and health, further exacerbate the economic situation of households [13]) ([14]).

Malaria: mosquitoes life cycle and transmission

The species *Anopheles gambiae* prefers to feed on humans indoors, especially at night, and is responsible for transmitting *P. falciparum*. Female mosquitoes require a blood meal to produce eggs, which are laid in water [15]. The eggs hatch into larvae, transition to pupae, and eventually emerge as adult mosquitoes [16].

Inside the adult mosquito, the parasite begins a sporogonic cycle lasting about 14 days. Within an hour after ingestion, gametocytes develop into gametes and fuse to form a zygote. Within 18 to 30 hours, the zygote transforms into a motile ookinete that crosses the peritrophic matrix and penetrates the mosquito midgut epithelium. The ookinete forms an oocyst, which divides

over 1–2 weeks to release sporozoites into the salivary glands, enabling transmission during the next blood meal [17] [18], [19] [20], **(Fig 1- A, B**).

In humans, sporozoites travel through the bloodstream to reach the liver, where they invade hepatocytes. Inside these liver cells, the parasites develop into schizonts, eventually maturing into merozoites (**Fig 1- A**). Merozoites invade red blood cells, replicate asexually, and cause cell destruction. Some differentiate into gametocytes, enabling transmission when taken up by a mosquito [21] [22].

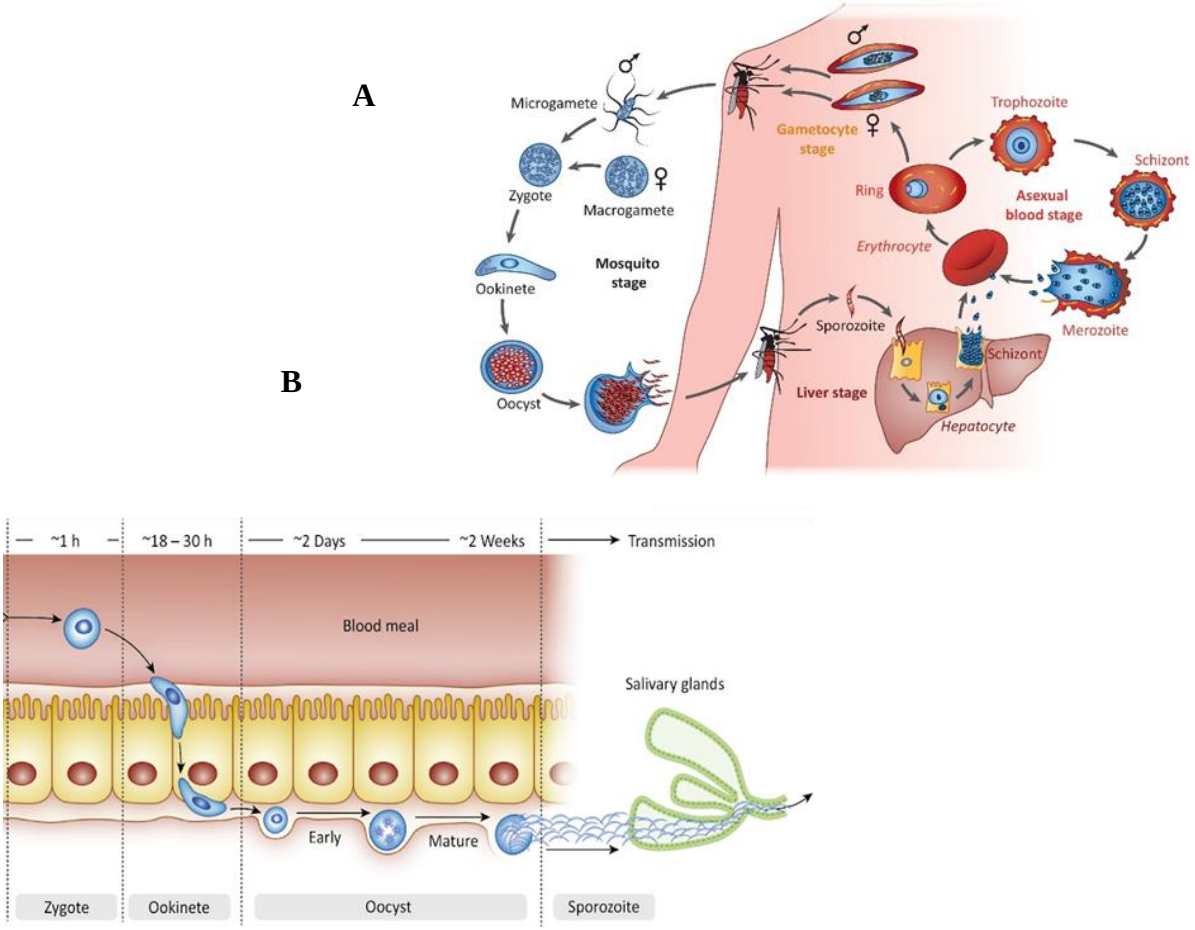

Figure 1 : **Malaria life cycle and transmission in human and mosquitoes (A)** (reproduced from the paper of [23] **and malaria parasite development in the mosquito (B)** (reproduced from the paper of [17])

Malaria: challenges and eradication

Targeting the parasite

The parasite's life cycle involves complex stages in vertebrate hosts and mosquito vectors, with both sexual and asexual reproduction, complicating drug and vaccine development [24]. Quinine was an early treatment but faced challenges in large-scale prevention [25] [26]. Artemisinin, isolated in 1970, became a key antimalarial agent [27] but is now facing resistance in the Greater Mekong region [28] [29] [30]. Underscoring the urgent need for new, durable treatments [29].

The parasite's resistance to antimalarial treatments has made the development of vaccines essential. Despite the complexity of the parasite and human immune responses [31] [29], two vaccines, RTS,S/AS01 (in 2021) and R21/Matrix-M (in 2023), have recently been approved by the WHO for children in areas of high transmission in sub-Saharan Africa, marking a key advance against malaria [32] ([33]. The RTS,S/AS01 and R21/Matrix-M vaccines face several limitations. They target only the sporozoite stage of *Plasmodium falciparum*, failing to block transmission or protect against other species like *P. vivax*, limiting their utility in multi-species regions [34]. Their efficacy is low (30–50%), declines over time, and requires booster doses for sustained protection, posing logistical challenges, especially in rural areas with weak infrastructure [33]. The RTS,S/AS01 vaccine, requiring four doses, complicates adherence for families and health systems [34]. Partial efficacy may create false security, undermining the use of complementary prevention tools like insecticide-treated nets and mosquito control, thereby impacting broader malaria control efforts.

Targeting the vector

- **Larvae stage**

According to the World Health Organization (WHO), the only intervention capable of significantly reducing malaria transmission, even from very high levels, is mosquito control, which can bring it down to close to zero [35]. Vector control mainly targets larvae and the adult stage of mosquitoes.

Larval management basically requires optimizing the human environment through planned urbanization, improved architecture, and minimizing stagnant water around habitats. Agricultural activities like market gardening and irrigated rice fields, often larval reservoirs,

must also be addressed [36] [37]. These measures rely on public policies and government action but are often constrained in Africa by limited economic resources [38].

Managing mosquito breeding sites is particularly difficult in rural and tropical areas, where vectors exploit scattered water sources such as puddles, tree holes, and artificial containers [38]. Though agents like *Bacillus thuringiensis israelensis* (Bti) and insect growth regulators are effective, their short residual activity and operational constraints limit widespread use [39]. Community-based efforts, such as eliminating stagnant water and applying larvicides are promoted by local volunteers to enhance awareness and participation [40], but sustained community engagement remains challenging.

- **Adult stage**

Vector control is mainly focused on adult mosquitoes, with the use of insecticide-impregnated mosquito nets at its heart. Their control was marked by the introduction of DDT in 1943, which was used on a massive scale after the Second World War and formed part of the WHO's eradication campaign in 1955. Abandoned in 1978 due to its effectiveness being insufficient [41]. It was replaced by pyrethroids, used to impregnate mosquito nets [42]. Although intensified control halved malaria deaths since 2000, progress has stalled, with around 500,000 deaths annually. [43] [4]. Mosquito resistance and adaptive behavior are undermining control measures, complicating malaria elimination efforts [41][35] [41]) ([42].

Insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) are the main malaria control methods recommended by the WHO [4]. Treated nets act as a barrier and kill mosquitoes on contact. Originally using pyrethroids, their efficacy dropped due to resistance.

A recent study [44] showed that users of long-lasting insecticidal nets had lower malaria infection prevalence and incidence than non-users, regardless of insecticide resistance levels. Given the increasing resistance of mosquitoes to insecticides, these products have been fortified, for example by adding piperonyl butoxide (PBO) to inhibit detoxification enzymes and enhance pyrethroid efficacy. However, bioassays have shown limited effectiveness in some parts of Africa [45]. As nets provide only partial protection, new vector control tools are needed to reduce the malaria burden.

Mosquitoes develop insecticide resistance through four main mechanisms, ranked by importance: (1) metabolic resistance, where detoxification enzymes degrade insecticide molecules; (2) target site resistance, involving mutations that reduce insecticide binding; (3) cuticular resistance, which limits insecticide penetration through the mosquito’s cuticle; and

(4) behavioral resistance, where mosquitoes avoid contact with treated surfaces [46]. Of these four mechanisms target site and metabolic resistances are most likely to lead to control failure [47] and a description of the target-site mutation, specifically the *kdr* mutation, will be provided in the section "Thesis Introduction".

Another current control tool: Gene drive technology

Faced with resistance to insecticides and anti-malarial drugs, genetic forcing technology offers a new approach. Genetic forcing technology is a strategy to combat malaria by altering mosquito genetics to reduce their capacity to transmit the *Plasmodium* parasite. This approach involves introducing specific genes into mosquitoes that limit transmission and promote the inheritance of these genes across populations [48].

Gene drive technologies raise technical and ethical concerns, notably their potential to spread uncontrollably, posing risks to ecosystems and biodiversity [49]. A key challenge in gene drive deployment for malaria control is ensuring strong governance. Coordinated efforts between national authorities, the WHO, and communities are essential to uphold safety and ethics. WHO prequalification requires robust data on safety, efficacy, and entomological impact before field use [50].

Understanding mosquito bioecology to improve vector control

The use of insecticides remains the cornerstone of vector control. However, the growing resistance of mosquitoes to these compounds is a major concern. Addressing this challenge it is critical to integrate knowledge of mosquito bioecology into control strategies. Understanding how key life-history traits such as longevity are influenced by environmental factors and linked to resistance phenotypes can inform more targeted and adaptive approaches. For example, mosquitoes with longer lifespans are more likely to transmit pathogens and may also exhibit enhanced resistance, particularly under favorable ecological or nutritional conditions. By incorporating ecological and physiological insights into resistance management, vector control programs can better predict resistance dynamics, tailor interventions to local contexts, and prolong the effectiveness of insecticidal tools.

1.2. Thesis introduction

In this thesis, I examined the influence of diet on mosquito longevity and response to insecticides. I explored how diets composed of plant nectar (*Thevetia neriifolia*, *Mandalium coromandelianum*, *Ixora coccinea*, *Tabernanthe iboga*, *Carica papaya*) or phytochemical metabolites (primary sugars: fructose, glucose, sucrose, trehalose; secondary metabolites: hydrogen peroxide, vitamin C, caffeine) impact resistance. Experiments included *Anopheles gambiae s.l.* (Tiassalé strain, resistant to all four classes of insecticide), *Anopheles coluzzii* (lab strain, resistant to pyrethroids) and *Plasmodium falciparum* (wild strain).

Mosquito longevity: a central feature in the epidemiology of malaria

Mosquito life-history traits such as larval development, fecundity, host-seeking behaviour influence their vectorial capacity. Among these, adult female longevity is the key factor in malaria transmission.

Indeed, the genus *Plasmodium*, the causative agent of malaria, requires an extrinsic incubation period (EIP) of 10 to 14 days within the mosquito before it becomes infectious [51] [52] [53]. Only mosquitoes that survive beyond this period can transmit the parasite to humans. Therefore, even a slight change in average mosquito longevity can cause disproportionate epidemiological effects, significantly increasing or decreasing transmission.

Longevity is shaped by various environmental factors, including access to sugar sources [54] [55] and infection status [56] [57]. Thus, while longevity is a biological trait, it also serves as a critical driver of malaria epidemiology.

Impact of adult mosquito's diet on their life history traits and malaria transmission

For malaria to develop, three elements are required: a vector (adult mosquito), a host and an environment favorable to transmission. The environment critically shapes vector-parasite interactions through biotic (flora, fauna, microbes), abiotic (climate, water), and anthropogenic (insecticides, pollutants) factors. Among these, biotic factors, particularly plant availability and accessibility, play a central role by shaping access to nutritional resources [58]. Indeed, the availability and quality of plant-derived sugars are major drivers of mosquito life-history traits [58]. Adult mosquitoes, including *Anopheles gambiae*, rely heavily on plant sources primarily floral nectar, but also leaves, stems, fruits, and extrafloral nectar to obtain the energy needed to perform all their vital functions [58] [59].

Plant-derived meals play an important role in mosquito life traits. For example, upon emergence, female mosquitoes prefer to feed on nectar rather than on blood [60]. Plant species subjected to mosquito diets influence their life traits such as longevity [61] [62] [63] [58] [64], reproductive rate [61], mating ability [64] and, above all, its biting behavior. Indeed, it has been shown that an environment rich in plant substances considerably reduces the mosquito's biting rate on the human host [65], and a 10% sugar supplement to the mosquito's blood diet reduced its vectorial capacity compared with those that received no sugar supplement [66].

Floral nectar is an essentially sweet solution [67][68] that facilitates interactions between plants and mutualists such as pollinators [67]. The pollinator role for *Anopheles gambiae* for instance is based on indirect observations indicating so far a negligible contribution to plant pollen transfer [69].

In addition to sugar, which consists mainly of sucrose, glucose and fructose, it contains proteins, amino acids, minerals and various secondary metabolite compounds [70]. Nectar constituents (quantity and quality) vary according to plant species and environmental conditions [71][72]. In addition to attracting pollinators, nectar also influences interactions with microbes, nectar thieves, and herbivores, and can manipulate the behavior of nectarivores [72]. It has been shown that disaccharides such as glucose and trehalose are the sugars most preferred by mosquitoes over monosaccharides [73]. Other studies highlight the toxicity of certain sugars such as arabinose, lactose and cellulose in reducing mosquito longevity [74]. Certain vitamins with roles similar to secondary metabolites increase mosquito longevity and mating ability [75]. Supplementation of sucrose solution with minerals and vitamins [76] increased mosquito longevity. Female *Aedes aegypti* prefer sucrose-based diets containing low concentrations of alanine, leucine, asparagine, tyrosine or histidine to sucrose alone [73]. Phytochemicals such as p-coumaric acid and quercetin upregulate genes involved in the regulation of longevity and xenobiotic metabolism, thereby extending mosquito lifespan [77].

Plant-based diets modulate the prevalence, infection intensity and development time of *Plasmodium falciparum* in mosquitoes. Some plants reduce or promote parasite transmission, underlining their key role in malaria dynamics. These interactions potentially influence the ability of mosquitoes to spread the disease, notably via peridomestic plants [63]. Ricin has been shown to accelerate parasite growth rates with earlier salivary gland invasion in mosquitoes [78]. Some plant nectars contain secondary metabolites that can limit insect infection by pathogens [79].

Resistance of mosquitoes to insecticide and pleiotropic effects of resistance genes

Insecticide resistance remains a major challenge in controlling vector-borne diseases. One key mechanism is metabolic resistance, where mosquitoes neutralize insecticides through elevated activity of detoxification enzymes mainly esterases, cytochrome P450s, and glutathione S-transferases which degrade the compounds before they can affect the nervous system [46]. Another key of insecticide resistance mechanism is target-site resistance through mutations that render the neuronal targets of the insecticide less sensitive to the active ingredient [4]. Insecticide resistance via target-site mutations includes *ace-1* (resistance to carbamates and organophosphates) and *kdr* (resistance to pyrethroids and DDT). The mosquitoes in this study carry the *kdr* mutation, a mechanism detailed in **(Fig 2)**. Insecticide resistance imposes physiological costs on mosquitoes. Indeed, resistance due to elevated detoxification enzyme production depletes mosquito energy reserves, limiting resources for other biological functions and creating trade-offs with key life-history traits. For instance, the overproduction of detoxification enzymes, such as esterases reduces energy reserves by up to 30%, limiting resources for other life-history traits [80]. These trade-offs reflect the pleiotropic effects of resistance genes [80]. Infection status and resistance genes also impose a cost on mosquito life-history traits [81] [82].

knock down resistance is a mutation of voltage-dependent sodium channels, which are the target of pyrethroid and organophosphate.

Normally (A-1) these insecticides bind to voltage-gated sodium channels in neuronal membranes. These channels regulate the passage of sodium ions necessary for the transmission of nervous signals. The insecticide prolongs the opening of the channels, disturbing the ionic balance. This leads to continuous depolarisation of the neuronal membrane, preventing action potentials from resetting. The result: the nervous system is paralysed and the mosquito loses motor control, leading to paralysis ("knockdown" effect) followed by death. (A-2) kdr mutation prevents pyrethroids and organochlorines from attaching to sodium channels, which makes the insecticides less effective and the neurons continue to function normally.

Impact of the environment on the expression of resistance

Insecticide resistance in mosquitoes is often considered to be genetically based, yet environmental factors have a significant impact on their range. Abiotic, biotic and artificial xenobiotic environmental factors all contribute to the selection of mosquito resistance to insecticides.

On the abiotic side, studies have focused more on temperature, with several studies providing evidence that high temperatures during mosquito larval rearing decrease the sensitivity of adult *Anopheles gambiae* sl. mosquitoes to pyrethroids and increase the expression of metabolic enzymes [83]. However, in the adult stage, the higher the temperature, the more sensitive mosquitoes are to insecticide exposure [84] [85].

From a biotic point of view, the feeding status of mosquitoes is a determining factor in their response to insecticides; for example, significant differences in the mortality rate of mosquitoes exposed to pyrethroids have been observed between starved and sugar-fed mosquitoes [86] [84]. The response of mosquitoes to insecticides also depends on plant nectar-based nutrient sources, as demonstrated by a single study to date [87]. The secondary metabolites present in nectar also have an effect on detoxification enzyme production, as in the case of p-coumaric acid and quercetin [77]. Other substances, such as alkaloids like caffeine, affect the nervous system of insects, causing neurotoxic effects similar to those of some insecticides, [86] [88] [89].

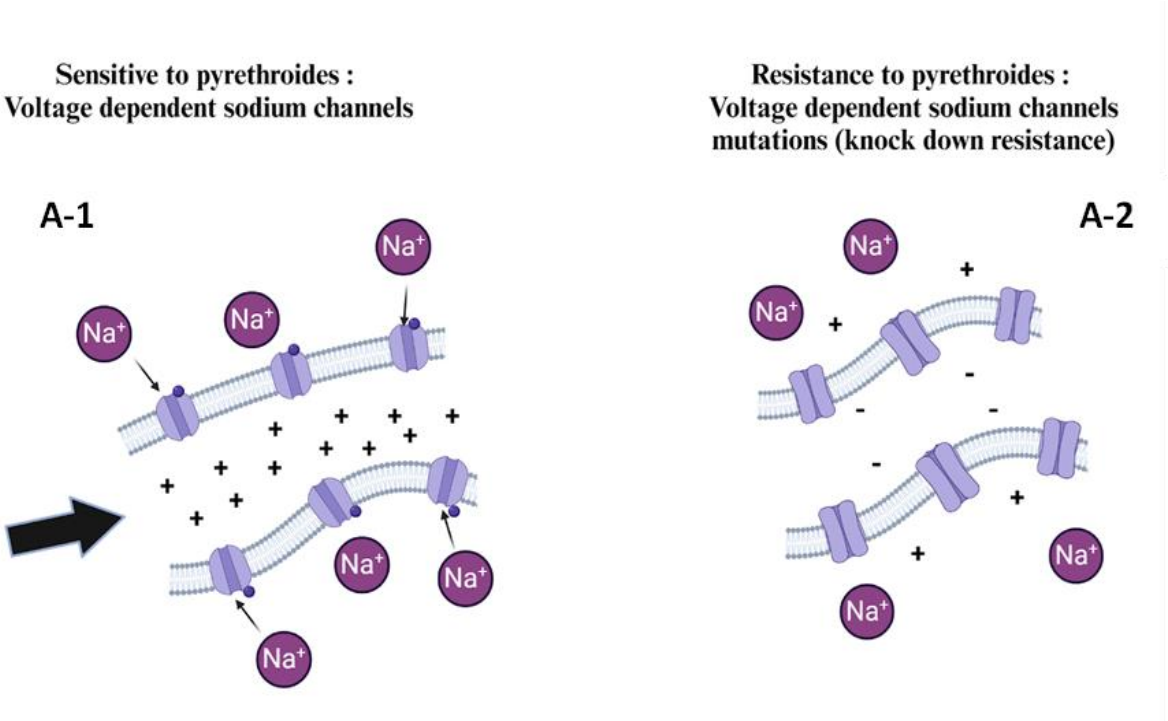

Figure 2 : **Mechanism of resistance in mosquitoes by modifying target sites (knockdown resistance)** (created with BioRender.com)

1.3. Problematics

In addition to taking blood-meals, mosquitoes regularly feed on the nectar of plants. The nectar, in particular the sugar it contains, serves as their main source of energy. Since the nectar diet is one of the main environmental factors influencing the life traits of mosquitoes, it is reasonable to think that the quality and quantity of nectar, which varies according to plant species, could also affect key parameters such as longevity and resistance to insecticides. These two parameters are particularly important for malaria epidemiology, as they condition the ability of mosquitoes to transmit malaria. However, to date, few studies have examined the impact of diet on these parameters. Several questions remain unanswered:

- Does longevity vary according to the plant species used? If so, how can this variation be explained, taking into account both the quantity of nectar ingested and the concentration of total sugars?
- How do both the blood meal and the plant-based diet influence the resistance of mosquitoes to insecticides?
- How the concentrations of certain key substances present in nectar (such as sugars, pro-oxidants and antioxidants) modulate the response of mosquitoes to exposure to insecticides?
- How does diet interact with the mosquito's infectious status to influence both insecticide resistance and post-exposure longevity?

These questions form the main line of my thesis, and the various chapters are devoted to them. They are also summarised in (**Fig 3)**.

1.4. Research aims

We studied the response of mosquitoes to insecticide and their longevity according to their diet. First, we investigated the effects of nectar and sugar on the longevity of the mosquito *Anopheles gambiae* s.l. **(chapter 2)**. In this chapter we aimed to understand how the quantity of nectar consumed and the concentration of total sugar could affect the longevity of mosquitoes. We then studied the effects of the diet of the mosquito *Anopheles gambiae* s.l. on its resistance to deltamethrin **(chapter 3)**; In this chapter, two experiments were conducted: The first focused on sugar meals taken from different plants and on the time between the blood meal and the exposure to the insecticide and the second focused on sugar meals taken from different plants and on the effect of the age at the exposure time. In **(chapter 4)** we explored the effect of specific primary metabolites and phytochemicals of nectar on the resistance to deltamethrin of

the mosquito *Anopheles gambiae*. Finally, in **(chapter 5)** we were focusing on the effects of a caffeine-based diet on insecticide resistance and longevity in *Anopheles coluzzii* infected with *Plasmodium falciparum*. In this last study we focused on the response to the insecticide 48 hours after exposure and monitored the longevity of surviving mosquitoes.

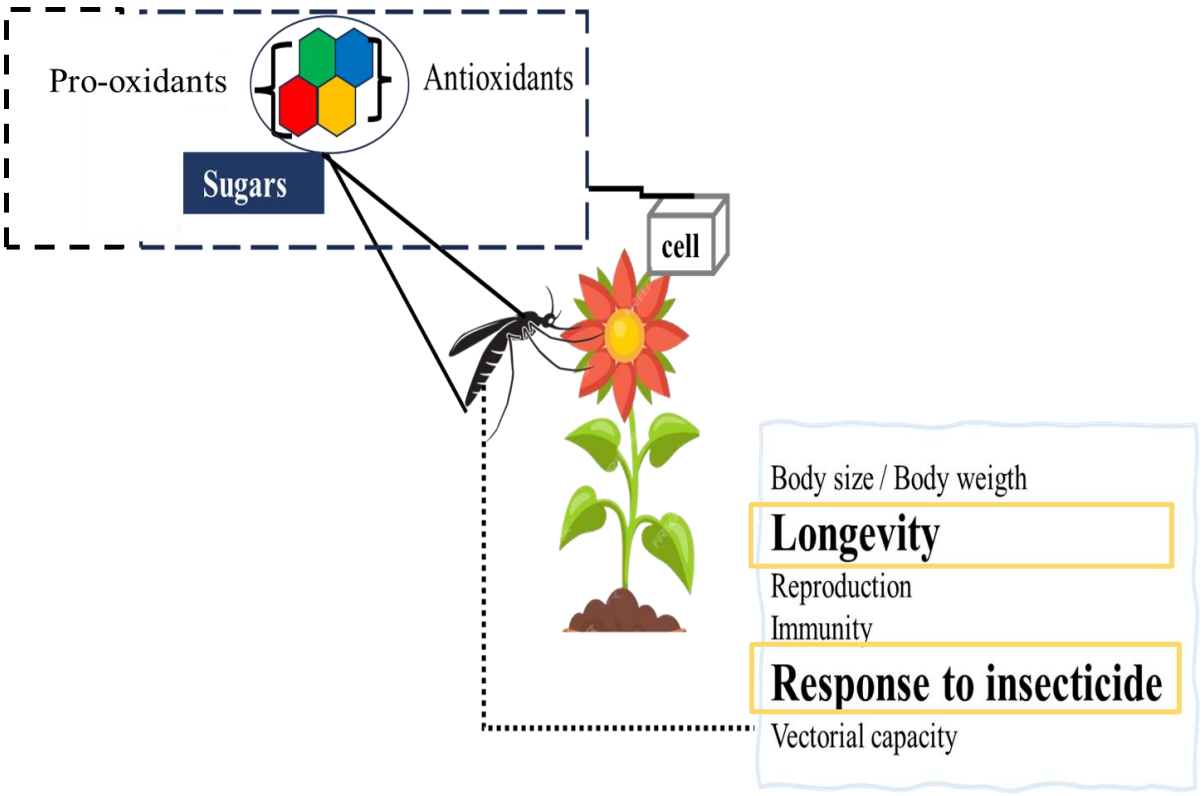

Figure 3: **Schematic summary of nectar-based diet affecting life history traits of mosquitoes** (personal elaboration)

Chapter 2: Effects of nectar and sugar on the longevity of the mosquito *Anopheles gambiae* s.l.

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2.1. Abstract

In addition to taking blood-meals, mosquitoes regularly feed on the nectar of plants. The nectar, in particular the sugar it contains, serves as a source of energy that underlies many life-history traits, including longevity. To understand better how different nectars and sugars influence the longevity of mosquitoes, we allowed adult *Anopheles gambiae* s.l. to feed on several plant species (the flowers of *Thevetia nerifolia*, *Mandalium coromandelianum*, *Ixora coccinea*, and *Tabernanthe iboga*, and the fruit of *Carica papaya*), measured the concentrations of sucrose, fructose and glucose in the nectar and fruit juice, estimated the size of their sugar meal and measured their longevity. The plant that the mosquitoes fed on affected their longevity (which ranged from an average of 8.2 days when they fed on *C. papaya* to 21.1 days when they fed on *M. coromandelianum*), and the mosquitoes took larger meals (in a separate experiment) from the plants that gave a longer life. However, longevity was only slightly affected by the concentrations of glucose, fructose, sucrose, or total sugar in the sugar meals. In contrast, when we let mosquitoes feed on experimental sugar solutions consisting of glucose, sucrose, fructose, or trehalose at concentrations of 1.97 or 19.97 kcal per 100 ml, both the type and the concentration of sugar affected longevity. The mosquitoes lived approximately one week longer when fed sugar at the higher concentration and they lived longest (14.1 days) when fed with sucrose and shortest (4.8 days) when fed with trehalose. Overall, our results show the importance of nectar and sugar on the longevity of mosquitoes but suggest also that non-sugar components of nectar may have a large impact.

keywords : longevity, *Anopheles gambiae*, nectar, sugar

2.2. Introduction

While female mosquitoes use blood meals for egg maturation, both sexes rely on various sources of sugar for survival after emergence [58] [90]. Therefore, the availability and quality of sugar sources strongly affect vectorial capacity of mosquitoes [58] [91] [66]. One of the main sources of sugar is the nectar of many plant species. Nectar is composed of different types of sugar (mainly sucrose, glucose, or fructose), amino acids, secondary metabolites, and microorganisms [67] [68]. Several studies have highlighted the impact of nectar on the life-history traits of mosquitoes, assuming that the impact of nectar is due to the sugar it contains [63] [59] [61] [64]. Since the type and concentration of sugars in nectar varies considerably among plant species [68], different plant species are expected to affect the life history traits of mosquitoes differently. However, there is limited information on how the size of the nectar meal and the types and concentration of sugar in nectar affect life history traits and, thus, the ability of mosquitoes to transmit infectious diseases [91].

One of the main traits underlying the transmission of diseases like malaria is longevity, for infected mosquitoes can transmit the parasite only if they survive long enough for it to reach its infective stage. Longevity is influenced by adult feeding, as the energy available at emergence is replenished by sugar sources [58]. Additionally, the longevity of *Anopheles gambiae*, the mosquito that transmits malaria, varies among the plant species it feeds on and it prefers to feed on the plant species that give the longest life [59] [61] [64].

To increase our knowledge of this important aspect of mosquito ecology, we asked whether the size of the nectar-meal and the concentrations of the sugars in the nectar influence the longevity of *A. gambiae* feeding on different plant species. First, we allowed mosquitoes to feed on the nectar or fruit juice of five plant species that grow naturally in Agboville, Côte d'Ivoire, and measured their longevity and the sizes of their nectar meals. We also measured the sugar concentrations in the nectar of each plant. Second, we let mosquitoes feed on different types of sugars at two concentrations and measure their longevity.

2.3. Methodology

The experiments were carried out in an insectary maintained at 25°C ± 2 °C, 76% ± 2 % relative humidity and a 12:12 h light: dark cycle, and with the offspring of *A. gambiae* that we collected as larvae from irrigated rice fields in the town of Tiassalé, Côte d'Ivoire, where most mosquitoes are highly resistant to all four classes of insecticides [92]. The larvae were brought to the insectary and reared to adulthood. Their offspring were reared in trays containing between 120 and 150 larvae in 700-800 ml of tap water and fed daily with Tetramin baby fish according to their age [93].

Plant-based diet

In a preliminary study, we tested whether mosquitoes fed on the flowers or fruit of 12 common species (**Table 1**), and selected for our experiments the five species on which mosquitoes were most likely to feed: the flowers of *Ixora coccinea*, *Malvastrum coromandelianum*, *Tabernanthe iboga*, and *Thevetia neriifolia*, and the fruit of papaya (*Carica papaya*).

Table 1: List of plant species used in the preliminary study

Plant species	Types of the plant species	Part of the plant used
Tabernanthe iboga	Ornamental	flower
Thunbergia erecta	Ornamental	flower
Ixora coccinea	Ornamental	flower
Costus woodsonii	Ornamental	flower
Allamanda cathartica	Ornamental	flower
Thevetia nerifolia	Ornamental	flower
Lantana camara	perennial	flower
Mandalium coromandelianum	perennial	flower
Aspilia africana	perennial	flower
Centraterum intermedium	perennial	flowers
Carica papaya	seasonal	fruits
Mangifera indica	seasonal	fruits

In the nectar or fruit juice of these five species we measured the total concentration of all sugars and the concentrations of glucose, fructose and sucrose. We extracted the nectar of the small flowers (*I. coccinea* and *T. iboga*) with a syringe, the nectar of *T. nerifolia* by pressing it into an Eppendorf tube. and the fruit juice of *C. papaya* by crushing the pulp, allowing it to settle, and then collecting the juice from the top. We could not extract nectar of *M. coromandelianum* because we did not see any nectar in the flower, even on the flower stems, a small amount of sticky substance was occasionally visible, but could not be collected. In each sample, we measured the concentration of fructose using a fructose assay kit (Sigma Aldrich, Saint Louise, USA; product code FA-20), reading the optical density with a spectrophotometer (absorbance at 340 nm) (Thermo Scientific, Genesys 10S UV-VIS). We then determined the proportions of fructose, sucrose, and glucose in the samples with nuclear magnetic resonance spectroscopy (Bruker Avance Neo Ascend 600MHz, Bruker, Germany; Mnova NMR software package v.14.2.0, MestReLab Research S.L., Spain) [94] and calculated the concentrations of glucose, sucrose and total sugar from the concentration of fructose.

For NMR analysis, 50 µL of each nectar sample was dissolved in 450 µL of D_2O. Spectra were acquired using a Bruker Avance Neo Ascend 600 MHz spectrometer (Bruker, Germany), employing the noesygppr1D pulse program for water-suppressed ^1H NMR spectra. A total of 256 scans were recorded at 25 °C. Spectral processing was performed with Mnova software (version 14.2.0, MestReLab Research S.L., Spain). Sucrose, fructose, and glucose were identified in good agreement with published reference data [95] [96] [94].

Male and female mosquitoes were kept separately in mesh-covered cages (30 × 30 × 30 cm). For each plant species we used nine cages for females and nine cages for males, each with 15 to 25 mosquitoes. Each cage contained one plant species as sugar source, either as a number of flowers that would provide about three ml of nectar (placed in a 500 ml Erlenmeyer flask filled with tap water, plugged with cotton wool, and sealed with Parafilm) or a papaya cut in half. Since for *M. coromandelianum* we could not extract and quantify any nectar, we provided 20 flowers.

We used three of the cages per plant per sex to measure longevity. From emergence onwards, the mosquitoes were given their sugar source, which was replaced daily. They were checked daily until all of them had died.

We used the other cages to estimate the size of the sugar meal. For the first 48 hours after emergence, we gave the mosquitoes water, and then placed them into cages with their sugar source and froze them at -20°C 18 hours later. We estimated the size of the sugar meal from the relationship between wet weight (measured to a precision of 1 μg) and wing length (measured from the distal end of the alula to the tip with the image-processing software ImageJ). To do so, we first found the mosquitoes that had no sugar in their gut with an Anthrone test [97], and calculated the linear relationship between their weights and wing lengths. We used this relationship to predict from their wing lengths the weight that a mosquito would have had, had it been unfed. The difference between the observed weight and the predicted weight (had it not fed) was used as a measure of the size of the sugar meal. We used the average size of the sugar meal taken from each plant species as a measure of preference for the species. Finally, we found the quantity of each sugar by multiplying our measure of sugar meal size by the concentration of each sugar.

Sugar-based diet

The sugar meal consisted of sucrose, fructose, glucose, or trehalose at a concentration of either 1.97 kcal or 19.7 kcal / 100 ml water, and was provided on a wad of cotton placed on top of the cage.

Female mosquitoes were kept in three mesh-covered cages per diet (15 × 15 × 15 cm), each with 15 to 22 mosquitoes. The sugar meal was renewed daily, and mosquitoes were checked daily until they had all died.

Statistical Analyses

The statistical analyses were performed with the software R version R-4.3.2. All analyses were linear mixed models, performed with the function lmer. We found the significance of the effects with the function Anova (package car), using a type 3 SS if the interactions were significant and a type 2 SS if they were not.

Plant-based diet: First, we assessed the impact of plant species on longevity with an analysis of variance that included plant species, sex and their interaction as fixed factors and cage as a random factor. We analysed the square-root of longevity so that the residuals were close to normally distributed. Second, we assessed whether the size of the sugar meal (i.e., the difference between the observed weight and the weight predicted for an unfed mosquito of the

same size) differed among plant species with an anova of the size of the sugar meal that included the same factors. Third, we assessed whether the size of the sugar meal affected longevity with an analysis of covariance of the square-root of longevity that included the average size of the sugar meal taken from a given plant species as a continuous variable, sex and their interaction, and cage as a random factor. We then assessed whether the sugar content of the plants affected longevity, ignoring data with *M. coromandelianum*, for which we had no nectar. We analysed the square-root of longevity first with an ancova that included the total sugar content of the plants, sex and their interaction, and then with an ancova that included the content of each sugar (fructose, sucrose and glucose), sex, and the interactions between sex and each sugar. Both analyses included the cage as a random factor.

Sugar based-diet: We analysed the impact of individual sugar diluted in water on longevity of female mosquitoes by including types of sugar, concentrations and their interaction as fixed factors, and cage as a random factor.

2.4. Results

Plant-based diet

The concentrations of total sugar in fruit juice of nectar of the plants (excluding *M. coromandelianum,* for which we could not extract any nectar) ranged from 30.1 g/l for *C. papaya* to 951.0 g/l for *T. iboga*. In the three nectars sucrose was the dominant sugar, but in the juice of *C. papaya* about a third of the sugar was fructose (**Table 2**).

Table 2: Concentrations (g/l) of sugars in the fruit of *C.papaya* and the three plants from which we could extract nectar.

Plant species	Sucrose	Fructose	Glucose	Total sugar
T. iboga	882	41	22.0	951
I. coccinea	466.7	7.0	3.5	500.0
T. nerifolia	96.2	3.0	2.5	107.1
C. papaya	0.1	12.6	10.3	30.1

The average longevity of the 500 mosquitoes (242 males and 258 females) was 15.9 days and ranged from 8.2 days (7.21-9.33) when the mosquitoes had fed on the fruit of *C. papaya* to 21.1 days on *M. coromandelianum* (19.1-23.3), (χ2=25.94, df=4, p<0.001) (**Fig. 1A**). Neither sex (χ2=0.42, df =1, p=0.516) nor the interaction between sex and plant species (χ2=2.55, df=4, p=0.635) affected longevity.

The average sugar meal size (so, the difference between the observed weight and the weight predicted for an unfed mosquito with the same wing length) ranged from 0.01 mg (-0.1-0.03) for males that had fed on *I. coccinea* to 0.21 mg (0.18-0.25) for females that had fed on *T. nerifolia*. Females took larger meals than males (χ2=64.5, df=1, p<0.001), and the size of the sugar meals differed among the plant species (χ2=41.29, df=4, p<0.001) (**Fig. 1B**).

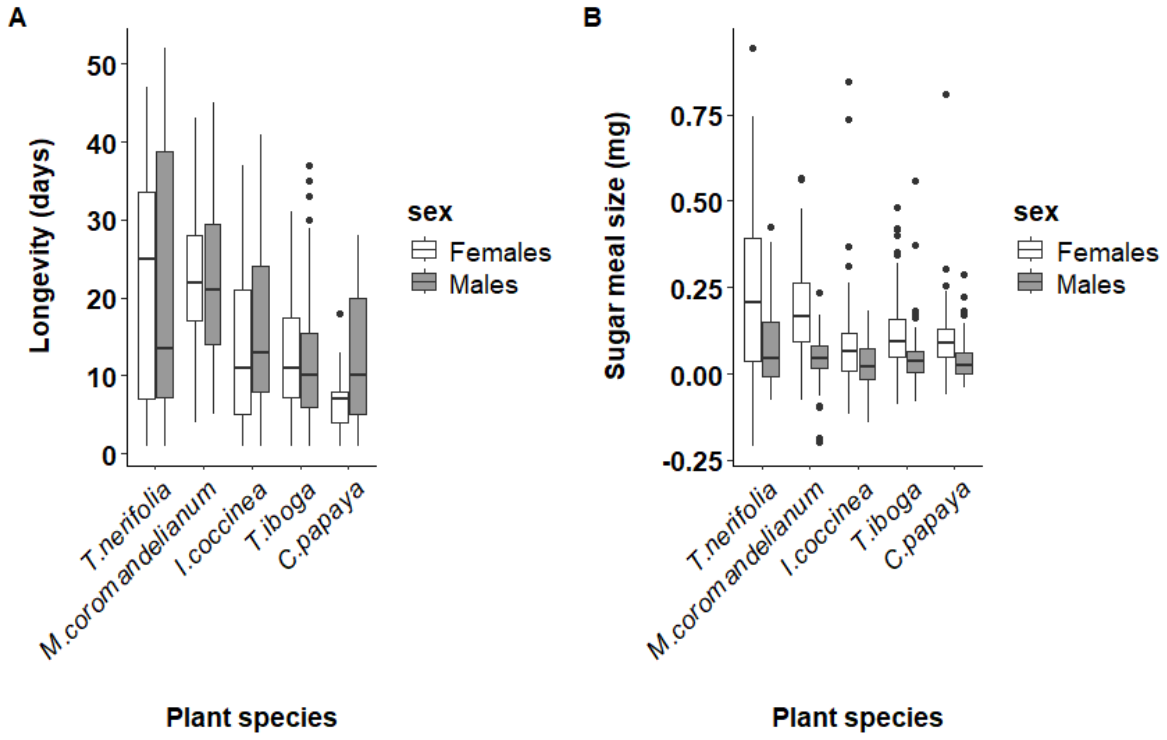

Figure 1: The effect of plant-based diet on the longevity (A) and (B) the size of the sugar meal of mosquitoes. The rectangle represents the interquartile range, that is, longevities between the 25th and the 75th percentile. The thick horizontal line within the rectangle denotes the median value. The vertical lines span four times the interquartile range, and the dots show outliers that are beyond this range.

The effect of total sugar concentration has a slight effect on the longevity of mosquitoes (χ2=11.33, df=3, p=0.010) but this effect was not influenced by sex (χ^2 = 0.63, df=3, p=0.427). Although the mean of longevity increased slightly between the lowest concentration of total sugar with *Carica papaya* [(30.07 g/l; 8.24 days (7.22-9.32)] and moderate concentrations with *T.nerifolia* [107.14 g/l; 18.74 days (15.4-22.4)], the mean of longevity did not follow a consistent upward trend at higher concentrations [with *I. coccinea* 500 g/l: 12.34 days (10.49-14.35); and *T.iboga* 952.55 g/l: 11.38 days (9.82-13.05). This non-linear pattern suggests that sugar concentration has only a limited effect on mosquito longevity (**Fig. 2**). Mosquitoes lived longer when they fed on plants from which they (in a separate experiment) took, on average, a larger sugar meal (χ2=12.94, df=1, p<0.001), whether the mosquitoes were males or females (interaction sex*meal size: χ2=0.01 df=1, p=0.916) (**Fig. 3**).

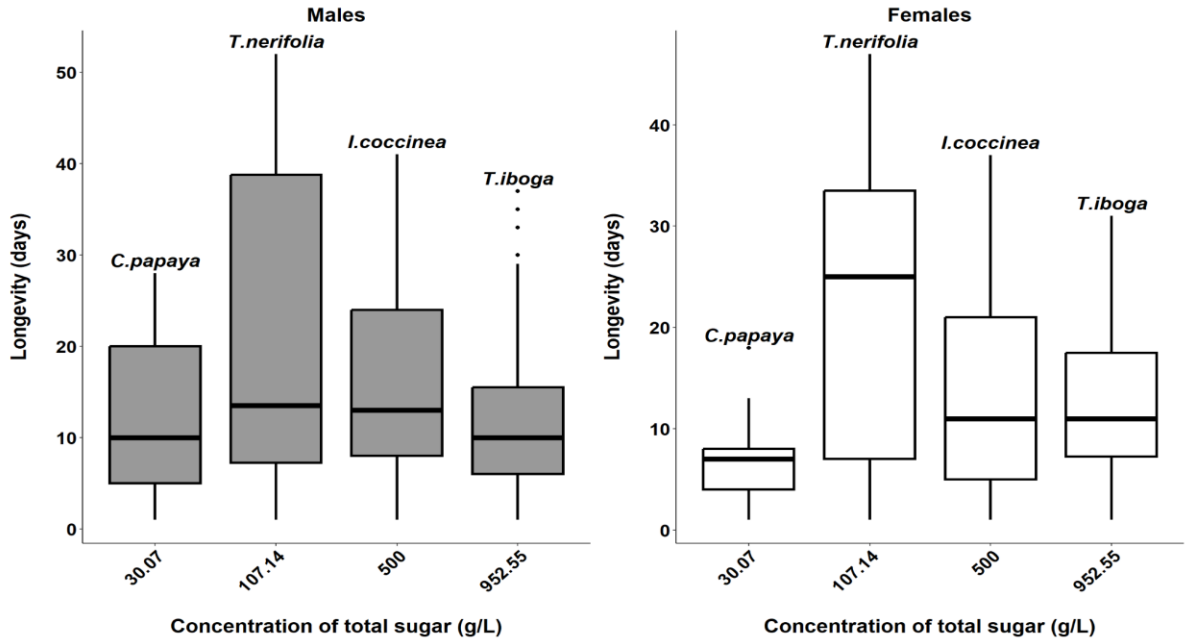

Figure 2: The effects of the concentration of total sugar in a plant sugar meal source on the longevity of mosquitoes. The rectangle represents the interquartile range, that is, longevities between the 25th and the 75th percentile. The thick horizontal line within the rectangle denotes the median value. The vertical lines span four times the interquartile range, and dots show outliers that are beyond this range.

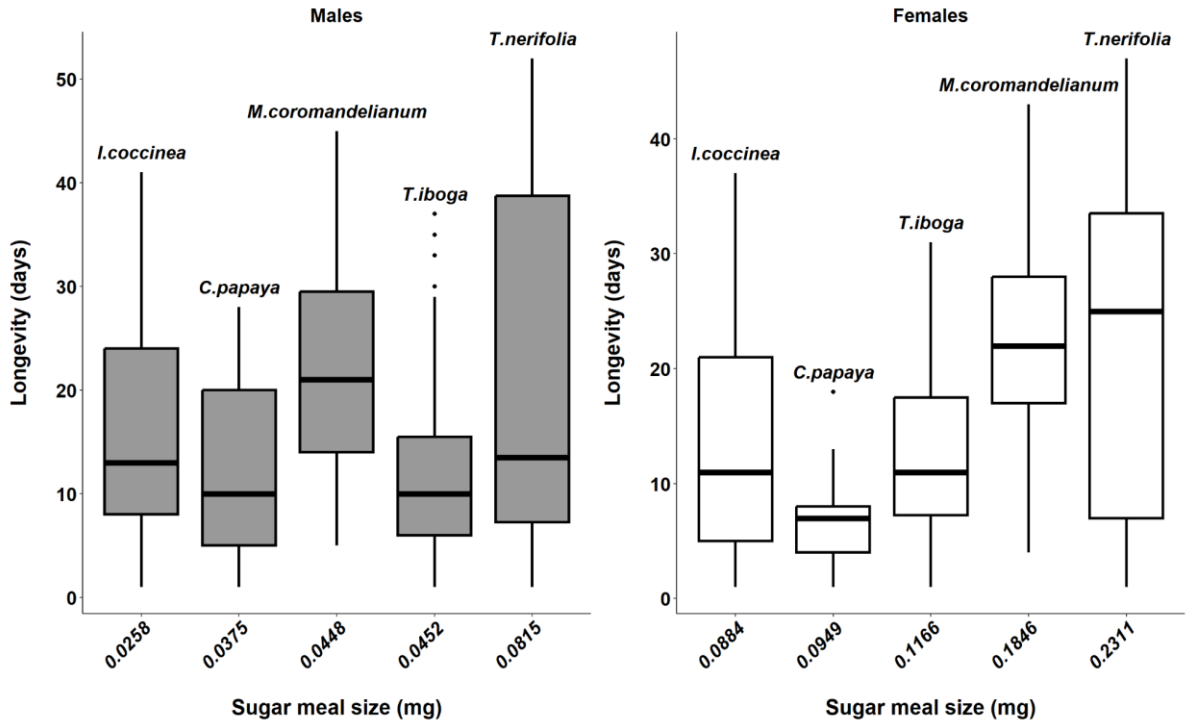

Figure 3: Relations between longevity and nectar meal size of mosquitoes feeding on different plant species. The rectangle represents the interquartile range, that is, longevities between the 25th and the 75th percentile. The thick horizontal line within the rectangle denotes the median value. The vertical lines span four times the interquartile range, and dots show outliers that are beyond this range.

Sugar-based diet

The average longevity of the 406 female mosquitoes was 11.4 days. The type of sugar had a significant influence on the longevity of the mosquitoes (χ2=29.76, df=3, p<0.001); longevity was 14.1 days (12.7-15.6 days) if the mosquitoes had fed on sucrose, 10.5 days (8.6-12.5) if they had fed on glucose, 11.7 days (10.3-13.2) if they had fed on fructose and 4.8 days (4.3-5.7) if they had fed on trehalose. The higher concentration increased longevity from 6.6 days (5.8-7.4) to 13.4 days (12.2-14.7) (χ2=25.99m df=1, p<0.001). There was an interaction between concentration and the type of sugar (χ2=19.10, df=3, p<0.001),

The effect of sugar concentration on longevity varied greatly depending on the type of sugar. For trehalose, an increase in concentration from 1.97 Kcal to 19.7 Kcal resulted in a marked increase in longevity from 2.3 (2.1-2.5) to 8.6 days (7.1-10.3) and for glucose in resulted in an increase from 4.1 (3.3-5.0) to 17.8 days (15.0-20.8). For fructose and sucrose, the effect of

concentration is more moderate (fructose: 11.3 (9.9-12.8) to 12.1(12.5-15.1), sucrose: 12.8 (11.0-14.6) to 15.4 (13.3-17.6), (**Fig. 4**).

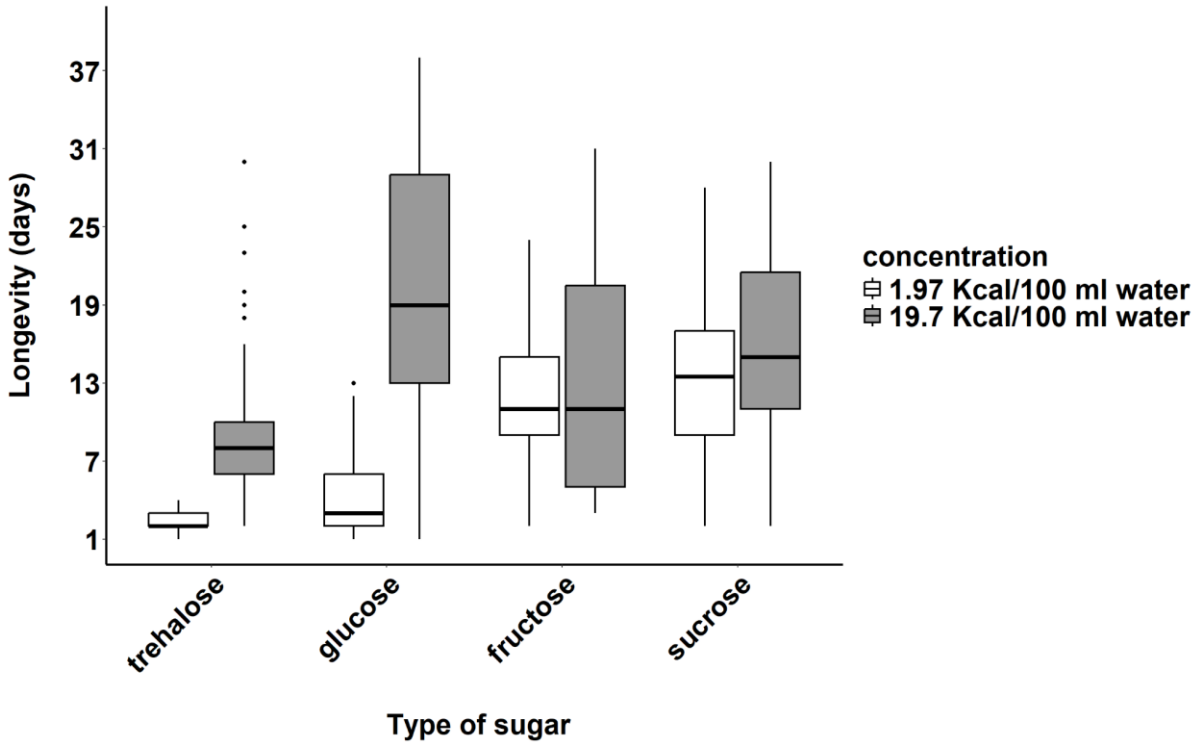

Figure 4: Effects of types and concentrations of sugar on the longevity of mosquitoes; The rectangle represents the interquartile range, that is the longevities between the 25th and the 75th percentile. The thick horizontal line within the rectangle denotes the median. The vertical lines span 1.5 times above the 75th percentiles and 1.5 times below 25th percentiles and the dots show outliers that are beyond this range.

2.5. Discussion

In our experiments the type and concentration of sugar in sugar-meals and the species of plant providing nectar strongly affected the longevity of *A. gambiae*. However, the concentration of sugars alone seems to exert a slight influence on longevity, suggesting that other components of nectar strongly influence longevity.

The concentration and type of sugar that mosquitoes feed on have strong consequences for their life-history [98] [99]. In our experiment, mosquitoes fed with the high concentration of sugar lived seven days longer than those fed with a 10-time lower concentration, and mosquitoes lived longer on sucrose than on the other sugars, in particular at the low concentration. This corroborates an experiment in which mosquitoes fed with sucrose accumulated more energy reserves than those fed with glucose or fructose [100] in energy supply and stress adaptation in mosquito hemolymph [101] [102] [103]. It corroborates, however, a study by Cissé-Niambélé, Koudou and Koella (in prep.), in which mosquitoes fed with trehalose were more likely to die after exposure to deltamethrin than those fed on other sugars. One reason may be that the metabolism of trehalose is not efficient if glucose is lacking, which leads to a deficiency of ATP [104] [105] [106].

The differences in longevity of the mosquitoes feeding on different plant species align with previous findings [59] [64,78] [107], in particular the long life when feeding on *T. nerifolia* [63] and the short life when feeding on *Carica papaya* [64,78].

The main component of nectar is sugar, the differences we observed among plants was slightly linked to their sugar content. Indeed, neither the total sugar concentration nor the concentration of the individual sugars explained strongly the difference in the longevity. While this result contrasts with studies showing a positive correlation between sugar content in plant parts and mosquito survival [61] result was found in *Conopomorpha sinensis*, where additional honey in the diet had no significant effect on longevity [108]. [61] suggest that other components than sugar, for example amino acids and secondary metabolites, play an important role in mosquitoes [77]. Indeed, the amino acids contained in nectar increase the longevity of female mosquitoes *Culex quinquefasciatus* [109].

The variation in nectar intake among plant species suggests that mosquitoes prefer some food sources over others. Such preferences can be linked to physical traits like the brightness of flowers or the shape of the corolla or the position of nectaries, which affect the access to nectar

[110]. They can also be linked to chemical emissions of the plants and nectars, which in turn can be linked to the quality of the nectar [61]. Indeed, plants that are preferred by mosquitoes provide more energy reserves than the less preferred [111]. This in turn leads to a positive correlation between nectar intake and longevity, for mosquitoes feeding on more nutritious plants benefit from increased sugar for energy and vital functions and therefore live longer [112] [113].

2.6. Conclusion

The longevity of mosquitoes varies from one plant species to another, although the concentration of sugar alone in the nectar does not strongly explain this longevity. However, different concentrations of sugar diluted in water have a considerable impact on longevity. This result highlights the fact that there must be other compounds in the nectar that have a major influence on the longevity of the mosquitoes observed.

2.7. Acknowledgements

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Chapter 3: Effects of the diet of the mosquito *Anopheles gambiae* s.l. on its resistance to deltamethrin

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3.1. Abstract

While we understand many aspects of the genetic basis of the resistance of mosquitoes against insecticides, the degree to which resistance is affected by environmental parameters like food remains unclear. We therefore focused on the diet of *Anopheles gambiae*, an important aspect of their environment, with two experiments investigating how sugar and blood meals affect their resistance to deltamethrin. The first focused on sugar meals taken from different plants and on the time between the blood meal and the exposure to the insecticide. Mosquitoes had continuous access to *Tevethia nerifolia*, *Ixora coccinea* or *Mandalium coromandelianum* as sugar meals, and half of the mosquitoes received a blood meal. After 15-18 hours (i.e., at a time when digestive genes are upregulated) or 60-63 hours (i.e., after digestion) we exposed them to 0.5 % deltamethrin for one hour and measured the proportion of mosquitoes that were knocked down during the exposure and that died within the next 24 hours. The plant species had no effect on the rates of mortality or knock-down. If the mosquitoes were exposed earlier, blood-feds were 22.7 % less likely to die and 10.0% less likely to be knocked down than unfeds, but if they were exposed later, blood-feeding increased mortality by 8.7% and knock-down by 14.0%. In the second experiment, we explored how the sugar-meal (consisting of the same three plants) interacted with the age at blood feeding. The mosquitoes were blood-fed or left unfed four or 11 days after emergence and exposed to the insecticide one day later. Neither the plant nor its interactions with blood meal or age affected mortality, but younger mosquitoes had lower mortality (60.7%) than older ones (66.4%), independently of their blood-meal. Similarly, the plant had no effect on knock-down rate, but the blood meal increased it by 14.5% in young mosquitoes and reduced it by 21.5% in old ones. These results underline the complex role of the mosquitoes' diet on their response to insecticides.

Keywords: nectar meal, blood meal, insecticide resistance.

3.2. Introduction

Insecticides, our main tool for the control of many mosquito-borne diseases, including malaria, are becoming increasingly ineffective due to the evolution of resistance [114] [115] [116]. However, it is not yet clear to what degree the spread of resistance genes affects the mosquitoes' vectorial capacity, for their effect on the response of a mosquito's life-history to exposure to insecticides depends on its age and its environment. Genetically resistant mosquitoes are, for example, more likely to be killed by insecticides as they get older [117], and whether they are killed is influenced by temperature [118].

An important aspect of the mosquitoes' environment is their diet, which consists of the nutrition during the larval stage and the blood meals and sugar meals taken by adults. Diet indeed affects the expression of resistance. Thus, mosquitoes that were well-nourished as larvae are more resistant than under-nourished ones as adults [117], and blood-fed adults are more resistant than unfed ones [119]. One reason why the diet influences resistance could be that resistance is energetically costly [80], so that a good diet (in particular the sugar meal) simply gives the mosquitoes more energy to survive the toxic effects of the insecticide. Another reason could be linked to the redox system and oxidative stress. Indeed, defense against oxidative stress is involved in insecticide resistance [120], so that inhibiting NADPH regeneration and thus enhancing oxidative damage with 6-aminonicotinamide decreases the ability of mosquitoes to detoxify insecticides and thus interfered with resistance [121]. Furthermore resistance can be linked to the activity of detoxifying enzyme families of esterases, glutathione-s-transferase, and cytochrome P450 [122], which also help mosquitoes to cope with oxidative stress. The redox system and oxidative stress, in turn, are directly linked to the diet. On the one hand, during their blood meals mosquitoes are exposed to high levels of reactive oxygen species [123], and on the other hand the nectar of plants ingested during sugar meals contains secondary metabolites that can act as prooxidants or antioxidants.

Thus, we expect that sugar meals should influence the resistance to insecticides, and indeed, plant-based diets can have a significant impact on mortality rates after exposure to insecticide [87]. Furthermore, since the concentration of sugar and secondary metabolites vary among plants, different plant species are expected to have different impacts on insecticide resistance.
To understand better the role of the diet of genetically resistant mosquitoes on their resistance phenotype, we considered in two experiments how the nectar obtained from different plant species, the availability of a blood meal, the mosquitoes' age (that is the timing of the blood

meal) and the time between the blood meal and the exposure to the insecticide affected the rate at which mosquitoes were knocked down or killed by the insecticide.

3.3. Methods

We collected larvae of the mosquito *Anopheles gambiae* in irrigated rice fields in the town of Tiassalé in southern Côte d'Ivoire, where most mosquitoes have a high level of metabolic resistance [114] [36] to all four classes of insecticides [92]. The larvae were brought to an insectary maintained at 25°C ± 2 °C, 76% ± 2 % relative humidity and a 12:12 h light: dark cycle, where they were reared to adulthood. Their offspring were used to carry out the experiment. These were reared in trays containing between 120 and 150 larvae in 700-800 ml of tap water, fed daily with Tetramin baby fish food according to their age (day of hatching: 7.2mg per tray; 1 day old: 10.8 mg ; 2 days old: 14.4 mg ; 3 days old: 28.8mg; 4 days old: 57.6 mg , 5 days old or older: 108 mg).

After emergence, females were distributed into cages (15 to 25 females per cage) and given continuous access to flowers of one of three plant species (*Thevetia nerifolia*, *Ixora coccinea* or *Mandalium coromandelianum*) as a source of nectar. We chose these species because we had found earlier that their nectar enables the mosquitoes to live a time that is similar to the longevity when fed on 5% sucrose, although the concentration of sugar differs strongly among the species [124]. The flowers were placed into a 500-ml Erlenmeyer flask that was filled with tap water, plugged with cotton wool, and sealed with Parafilm. For the species *T. nerifolia* and *I. coccinea* we provided the number of flowers that provided about 2 ml of nectar for the species *M. coromandelianum* we did not observe any nectar, so we provided 20 flowers. The plants were replaced every day.

Within each plant species, each cage was assigned randomly to the treatments (blood-feeding or not and timing of events). Thus, the mosquitoes in half of the cages were given a blood meal from a human arm. (The timing of the blood meals differed among experiments; see below.) Three hours before the time of the blood meal, the plants were removed from the cages. An experimenter who had avoided tobacco, alcohol and perfumed products for the previous 72 h offered his arm in a dark room to each group of mosquitoes for 20 minutes. From the blood-fed cages, the mosquitoes that were not fully fed were discarded.

Furthermore, at predetermined times after the time of the blood-meal (see below), the mosquitoes were exposed for one hour to filter papers impregnated with 0.5% deltamethrin according to the protocol of WHO [125]. We exposed the mosquitoes from a cage in a single WHO-tube, and then measured the likelihood that they were knocked down during the hour of the exposure and that they died within 24 hours of exposure.

This general design was used in two separate experiments to ask different questions (in addition to understanding the effects of the types of diet), and each experiment was replicated four times. In the first experiment we were interested in how the timing of exposure affects resistance. We therefore blood-fed mosquitoes when they were four days old, and exposed all of the mosquitoes to the insecticide either 15 to 18 hours or 60 to 65 hours later. In four replicates we exposed a total of 688 mosquitoes, so we were able to use an average of about 15 mosquitoes per cage.

In the second we were interested in how the age of the mosquitoes when they blood-feed affects resistance. We therefore blood-fed or did not feed mosquitoes either four days or eleven days after emergence. The mosquitoes were exposed to the insecticide the day after the blood meal. In four replicates we exposed a total of 663 mosquitoes, so about 14 mosquitoes per cage.

The statistical analyses were performed with R version R-4.4.2 [126]. All analyses were general linear models with a binomial distribution that included the cage the mosquitoes were held in as a random effect. We found the significance of the effects with the function Anova (package car) [127], using type 3 SS if the interactions were significant and type 2 SS if they were not.

In the first experiment we analyzed the knock-down rate and the mortality rate with models that included the plant species, the presence or absence of a blood meal, the time of the exposure, and all interactions as independent factors. In the second experiment we analyzed the knock-down rate and the mortality rate with models that included the plant species, the presence or absence of a blood meal, the age at which the mosquitoes obtained their blood meal and all interactions as independent factors.

3.4. Results

Experiment 1: Timing of exposure to insecticide

Mortality: Of the 688 mosquitoes 72.1 % died within 24 hours of their exposure to the insecticide. Mortality rates was similar for *I. coccinea* (75.3%; 95% confidence interval 68.2%-81.2%), *M. coromandelianum* (70.2%; 64.5%-75.3%) and *T. nerifolia* (72.0 %; 66.1%-77.2%) (χ2=0.3, df=2, p=0.869). However, mortallity was 11.1 % lower for blood-fed than for unfed mosquitoes (χ2=6.29, df=1, p=0.012) and 18.0 % lower for mosquitoes exposed to the insecticide late after blood-feeding than for those exposed early (χ2=19.22, df=1, p<0.001). The effect of the blood-meal depended on the time of exposure (interaction blood meal x time of exposure: χ2=23.53, df=1, p<0.001), with the blood-meal reducing mortality by 22.7% if the mosquitoes were exposed early, but increasing mortality by 8.8% if they were exposed late. There was no two-way or three-way interaction between the plant species and the blood meal or the time of exposure (all interactions: χ2<3.53, df=2, p>0.171) (**Fig1A**).

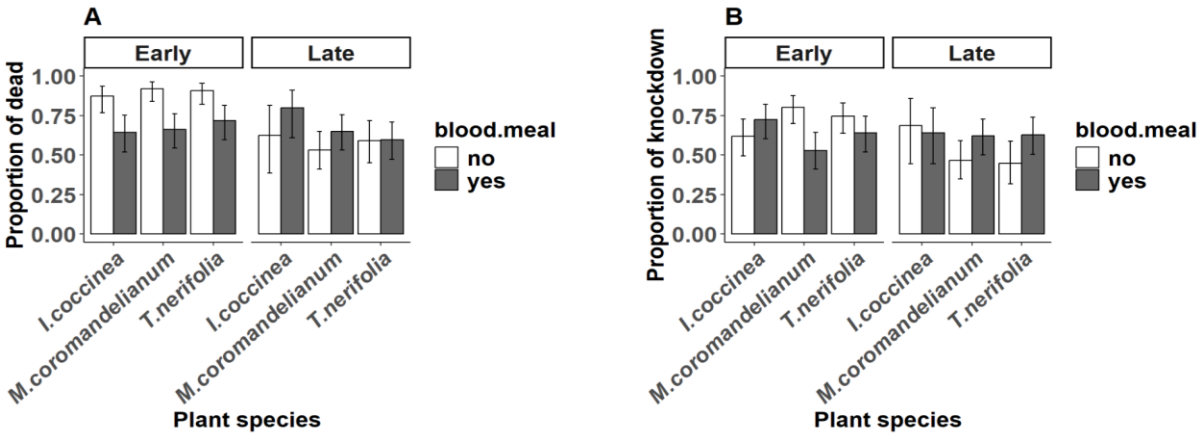

Figure 1: Effects of the sugar meal, the blood meal, and the time between the blood meal and the exposure to insecticide on their response to 0.5 % deltamethrin. A) Proportion of mosquitoes that died within 24 hours of exposure. B) Proportion of mosquitoes that were knocked down during the one hour of exposure. The error bars represent the 95% confidence intervals of the proportions.

Knock-down: 63.4 % of the mosquitoes were knocked down during the one hour of exposure. Knock-down rates were similar for *I. coccinea* (66.9%; 59.4%-73.6%), *M. coromandelianum* (61.4%; 55.5%-67.0%) and *T. nerifolia* (63.2%; 57.1%-68.9%) (χ2=1.09, df=2, p=0.579). The

percentage of mosquitoes that were knocked down was 11.7 % lower for mosquitoes exposed early after blood-feeding than for those exposed late after blood feeding (χ2=5.55, df=1, p=0.019). While the main effect of the blood meal was not significant (χ2=0.02, df=1, p=0.885), it interacted with the timing of exposure to affect knock-down rate (χ2=4.99, df=2, p=0.025), with the blood meal reducing the rate of knock-down if mosquitoes were exposed early by 10.0% but increasing it by 14.0% if they were exposed late. There was no interaction between the plant species and either the blood meal (χ2=1.75, df=2, p=0.415) or the timing of the exposure of insecticide (χ2=1.84, df=2, p=0.397), but the three factors interacted to affect knock-down (χ2=7.86, df=2, p=0.019). Thus, for example, the lowest knockdown rate of mosquitoes exposed early was found for blood-fed mosquitoes that had fed on *M. coromandelianum*, but the lowest rate of mosquitoes exposed late was found for unfed ones that had taken sugar from *T. nerifolia* **(Fig 1 B).**

Experiment 2: Age at blood-meal

Mortality: Of the 663 mosquitoes 63.1 % died within 24 hours of exposure. Mortality rates were similar for *I. coccinea* (62.3%; 54.3%-69.6%), *M. coromandelianum* (57.1%; 50.7%-63.3%), and *T. nerifolia* (68.5%; 62.8%-73.7%) (χ2=4.12, df=2, p=0.128). No interactions between plant species and blood meal or age at blood feeding were significant (χ2<3.76, df=2, p>0.152). The proportion of mosquitoes that died within 24 hours of exposure was 23.8% lower for blood-fed individuals than for unfed ones (χ2=35.30, df=1, p<0.001) and 5.8% lower for the younger mosquitoes than for the older ones (χ2=7.51, df=1, p=0.006). The two effects depended on their interaction, with blood-meal reducing mortality by 16.4% if the mosquitoes were young and by 35.4% if they were old (interaction blood meal x age χ2=5.29, df=1, p=0.021) (**Fig 2 A**).

Knock-down: 63.1 % of the mosquitoes were knocked down within 24 hours of exposure. The knock-down rates were similar for *I. coccinea* (64.2%; 56.3%-71.4%), *M. coromandelianum* (57.5%; 51.1%-63.7%), and *T. nerifolia* (67.0%; 61.3%-72.3%) (χ2=2.32, df=2, p=0.313). They were not affected by the interactions between the plant species and either the blood meal or the age at which mosquitoes received their blood meal (χ2<2.49, df=2, p>0.431). While neither age (χ2=0.62, df=2, p=0.431) nor blood meal (χ2=0.29, df=2, p=0.586) influenced the rate of knock-down, their interaction did, with blood-meal increasing knock-down by 14.5 % if the mosquitoes were young, but reducing knock-down by 21.5% if they were old (interaction blood meal x age χ2=17.16, df=1, p<0.001) Finally, there was an interaction between the three

factors (χ2=6.58, df=2, p=0.037). Thus, for example, the highest knockdown rate of mosquitoes fed early was found for blood-fed mosquitoes that had taken nectar from *I. coccinea*, but the highest rate of mosquitoes fed late was found for unfed ones that had taken sugar from *M. coromandelianum* (**Fig 2 B**).

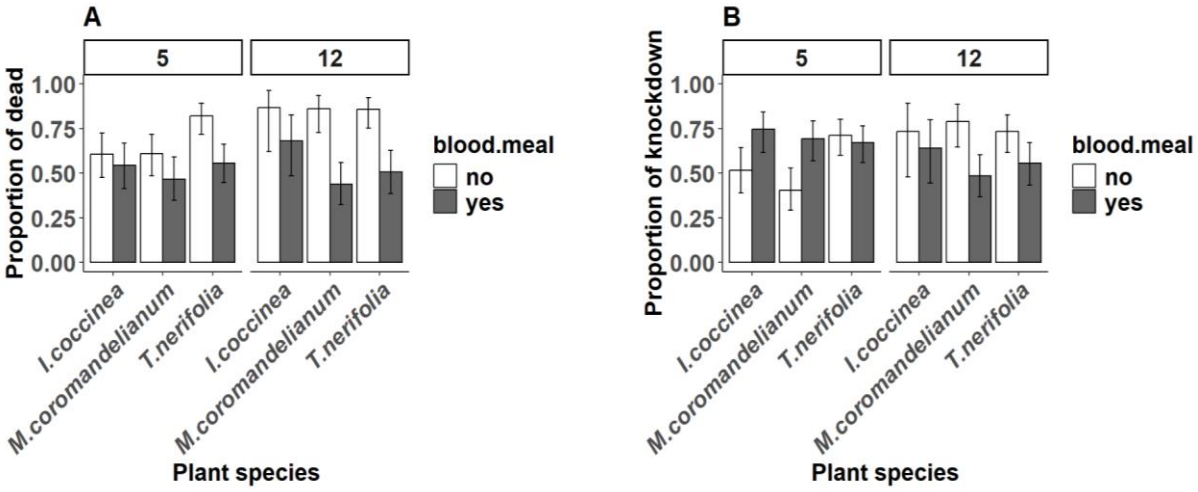

Figure 2: Effects of the sugar meal, the blood meal, and the age at which mosquitoes fed on blood on their response to 0.5 % deltamethrin. A) Proportion of mosquitoes that died within 24 hours of exposure. B) Proportion of mosquitoes that were knocked down during the one hour of exposure. The error bars represent the 95% confidence intervals of the proportions.

3.5. Discussion

While our experiments corroborated studies showing that blood meals and age affect the mortality of mosquitoes after exposure to an insecticide [128];[119], they contrast other work [87] in that the species of plant serving as a sugar source did not affect the rates of knock-down or mortality of mosquitoes within 24 hours of exposure to 0.5% deltamethrin, either as a main effect or in a two-way interaction with blood meal or age.

The difference between the two studies may be due to the fact that we used plants that gave similar longevity to a sucrose solution in preliminary studies, while the effects of the plants on unexposed mosquitoes is not described in Paré et al (2022). Indeed, it might be expected that in the latter study species giving high mortality (*Barleria lupulina*) may provide mosquitoes with little sugar and energy due to its antidiabetic activity though its impact on amylase [129]. Although our plants give similar longevities, we had expected differences in the mosquitoes' response to insecticides, for the concentrations of sucrose, fructose and glucose (Cissé and Koella, in prep) differ among the plant species we used [72] [68] [130][77], and such differences can translate to differences among life-history traits including longevity [77] [59]; [63]. The lack of difference between our plants suggests that other primary and secondary metabolites override the impact of sugar. While the sugars provide sufficient cumulative energy for longevity, they may fall short for the high-energy demands required for rapid detoxification of insecticides.

The increase in mosquito resistance to insecticides after a blood meal is likely due to the upregulation of detoxification enzymes, including esterases, glutathione S-transferases, and cytochrome P-450 [131] [132]. Indeed, the cytochrome P-450, for example, increases 14.5-fold one day after blood-feeding [132]. This is at least partly due to the reduction of the protein carbonyl content (a marker of oxidative stress) induced by the blood meal, which increases the activity of detoxification enzymes [120,133]. In our study the mosquitoes that had fed on blood 15-18 hours before being exposed to the insecticide were less likely to die than those exposed 60-63 hours after feeding. This corresponds to the peak of activation of the concentration detoxification enzymes after the blood meal [134] [135].

The higher mortality rate induced by exposure to an insecticide in older mosquitoes corroborates many studies [128] [136] [137] [138] [139] [140]. A possible reason is that the older the mosquito, the greater the increase in ROS caused by the increased activity of detoxification enzymes induced by increased energy metabolism. This age-related increase in

ROS metabolism may limit the ability of the older mosquito to catabolise the insecticide. These observations have been confirmed in flies and bees [141] [142]. Furthermore, in aphids, a negative correlation between antioxidant enzyme activity and their development was observed [143].

That blood-feeding decreased the effect of age insecticide resistance, could simply mean that blood meals replenish some of the reserves that are diminished with increasing age. The interaction between blood meal and age appears, however, to be complex, for in another study the opposite results was found, with a blood meal increasing resistance more in young than in old mosquitoes [128].

Our study has several limitations. First, the mosquitoes originated from a single, highly resistant population from Tiassalé and were reared in controlled laboratory conditions, which may limit the generalizability of the findings to other natural populations of *A. gambiae*. Second, only one blood donor was used throughout the study, so that our results may reflect a response specific to that donor. Third, only one insecticide (deltamethrin) was tested so, the results obtained do not allow extrapolation to other classes of insecticide to which resistant mosquitoes could react differently. Finally, the study focused solely on knock-down and 24-hour mortality, which does not capture sublethal or long-term effects that may influence malaria transmission.

3.6. Conclusion

Overall, although the plant species *T. nerifolia*, *I. coccinea*, and *M. coromandelianum* differ markedly in the sugar content of their nectar, feeding on them resulted in similar 24-hour mortality rates after exposure to 0.5% deltamethrin. This suggests that other nectar constituents may play a role in influencing mosquito susceptibility to insecticides.

These findings highlight the complexity of resistance phenotype and the importance of integrating plant management into vector control programmes, also considering mosquito diet when assessing resistance levels in field-based bioassays.

3.7. Acknowledgements

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Chapter 4: Impact of sugar and specific phytochemicals in the diet of *Anopheles gambiae* sl. on its resistance to deltamethrin

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4.1. Abstract

Nectar is the main energy source for mosquitoes, and its phytochemicals contribute to the physiological maintenance of nectar-feeding insects. However, their role in shaping insecticide resistance phenotypes remains poorly understood. To address this, we investigated how specific dietary components affect resistance in *Anopheles gambiae* sl.

We conducted three experiments in which female mosquitoes were fed on their assigned diets from emergence until 4-5 days old, then exposed to 0.25% or 5% deltamethrin in WHO tube tests.

In the first experiment, mosquitoes were fed on sucrose, glucose, fructose, or trehalose at low (1.97 kcal/100 ml of water) or high (19.7 kcal/100ml of water) concentrations. Mortality was highest in mosquitoes fed trehalose (84.1 %), followed by fructose (74.1 %), sucrose (67.9 %), and glucose (67.1 %). Lower concentration resulted in higher mortality (87.1 %) than higher concentration (59.2 %). No interaction between sugar type and concentration was detected.

In the second, mosquitoes were fed on 10% glucose supplemented with 0, 50, or 200 ppm caffeine. Mortality increased with caffeine concentration, from 11.4% (0 ppm) to 15.3% (50 ppm) and 41.0% (200 ppm).

In the last experiment, they fed on 10% glucose with or without vitamin C and/or hydrogen peroxide. Mortality was higher when mosquitoes fed on hydrogen peroxide (38.9%) compared to those fed on sugar without any supplement (20.4%). And the lowest mortality rate was recorded with vitamin C (10.0%). No interaction between the vitamin C and hydrogen peroxide was detected.

Overall, our study highlights the potential of specific phytochemicals to enhance insecticide efficacy.

Keywords: Insecticide resistance, nectar, phytochemicals, mosquito diet.

4.2. Introduction

Oxidative stress, an inevitable outcome of aerobic metabolism, plays a central role in the physiology of mosquitoes and other aerobic organisms by maintaining cellular homeostasis through the balance between reactive oxygen species (ROS) and antioxidant defenses [120]. Diet influences the availability of pro-oxidant and antioxidant compounds, thereby modulating the oxidative balance that supports essential physiological processes such as energy production and detoxification in mosquitoes [144] [128] [145].

Mosquitoes rely on a diet primarily based on nectar, their main source of energy, whose availability and composition influence key biological traits such as longevity [117] [91] [58]. Nectar not only provides sugar, but also contains a variety of primary and secondary metabolites and some of these phytochemicals act as antioxidants, while others have pro-oxidant properties. It also contains secondary metabolites such as phenolics, alkaloids, terpenes, and saponins, of which some act as plant defenses and are toxic to insects [146]. Additionally, metabolic by-products like hydrogen peroxide and superoxide, which have antimicrobial properties and help plants cope with environmental stress [147,148] [149] [150], as well as vitamin C, which neutralizes reactive oxygen species like H_2O_2, stabilizes nectar and limits oxidation and microbial growth [151] [152] [153].

Differences in nectar chemistry among plant species can affect mosquito life traits in distinct ways. For instance the longevity, reproduction fitness, fecundity, varies from one species to another one [59] [64,78][59][64,78] [107], also the resistance to insecticide is affected by different plant species [87]. These observations suggest that individual chemical components present in nectar are likely to differentially impact mosquito physiology, life-history traits, and responses to insecticides.

Oxidative stress has been closely linked to insecticide action and resistance. In mosquitoes, insecticide exposure generates reactive oxygen species (ROS), and the ability to manage this oxidative challenge may influence both survival and resistance phenotypes. One example is that dietary quercetin and p-coumaric acid have been shown to upregulate cytochrome P450 enzymes, which are also involved in insecticide resistance [77]. It is therefore plausible that nectar-derived compounds, by modulating oxidative balance, could directly or indirectly alter mosquito responses to insecticides.

However, the specific contributions of sugar types, concentrations, and bioactive nectar phytochemicals to insecticide susceptibility remain poorly explored.

Here, we assess how the type and concentration of sugar, and several important secondary compounds like vitamin C, hydrogen peroxide, and caffeine affect the susceptibility of *Anopheles gambiae* to deltamethrin.

4.3. Methods

The experiment with sugar was conducted by using the strain *Anopheles gambiae* collected from irrigated rice fields in Tiassalé (southern Côte d'Ivoire), where most mosquitoes have several resistance genes and are highly resistant to all four classes of insecticides [92]. The larvae were brought to the insectary and reared to adulthood. Their offspring were reared in trays containing between 120 and 150 larvae in 700-800 ml of tap water and fed daily with Tetramin baby fish according to their age [93].
The last both experiments were conducted at the Institut de Recherche en Sciences de la Santé (IRSS) de Bobo Dioulasso, in Burkina Faso. We used the *Anopheles coluzzi* lab strain from Kou valley (11°24′N, 4°24′59″W), which exhibits metabolic resistance mediated by cytochrome p450 and target-site resistance mediated by the kdr gene.
All mosquitoes of these experiments were maintained in a biosafety room at 27 ± 2 °C, 70 ± 5 % relative humidity and 12:12 light-dark cycle.

In the first experiment, mosquitoes were fed on sucrose, fructose, glucose, or trehalose at concentrations of 1.97 or 19.7 Kcal/100 ml from emergence until 5 days old. Afterward, we exposed the mosquitoes from a cage in a single WHO-tube, and then measured the likelihood that they were knocked down during the hour of the exposure and that they died within 24 hours of exposure.
In the second experiment, mosquitoes were fed on a 10% glucose solution supplemented with either 0 ppm, 50 ppm, or 200 ppm of caffeine from emergence until 4 days old. Afterward, we exposed the mosquitoes from a cage in a single WHO-tube, and then measured the likelihood that they were knocked down during the hour of the exposure and that they died within 24 hours of exposure.
In the last experiment, they were fed on a 10% glucose solution supplemented with either vitamin C (1 mg/ml), hydrogen peroxide (8 mM), a combination of both, or no supplement from emergence until 4 days old. Afterward, we exposed the mosquitoes from a cage in a single WHO-tube, and then measured the likelihood that they were knocked down during the hour of the exposure and that they died within 24 hours of exposure.

The statistical analyses were performed with R version R-4.4.2 [154]. All analyses were general linear models with a binomial distribution that included the cage the mosquitoes were held in

as a random effect. We found the significance of the effects with the function Anova (package car) [155], using type 3 SS if the interactions were significant and type 2 SS if they were not.

Each experiment was replicated four times. In the first experiment, the type, the concentration of sugar and its interaction were fixed, independent factors. In the second experiment the concentration of caffeine was considered as the fixed effect. In the last one, vitamin C and hydrogen peroxide were considered as fixed effects and all interactions as independent factors.

4.4. Results

Type and concentration of sugar

We tested 1,280 female mosquitoes fed on different sugars and concentrations, 72.3% died 24 h after 0.5% deltamethrin exposure. The type of sugar had a significant influence on the mortality rates within 24 hours after exposure (χ2=33.13, df=3, p<0.001). From the highest mortality rate to the lowest, we have trehalose with 84.1% (78.7%-88.3%), fructose 74.06% (69.4%-78.2%), sucrose 67.9% (62.9%-72.4%) and glucose with 67.1% (61.7%-72.0%). The concentration of sugar also had a significant influence on the rates of mortality (χ2=137.99, df=1, p<0.001). Among mosquitoes fed on the lowest sugar concentration, 87.1% (84.2%–89.5%) died within 24 hours, compared to 59.2% (55.4%–62.8%) at the highest concentration. There was no interaction between sugar concentration and the type of sugar (χ2=7.18, df=3, p=0.066), (**Fig 1A**).

60% of mosquitoes were knocked down within 1 hour of insecticide exposure. The knock-down rates significantly influenced by sugar type, (χ2=12.82, df=3, p=0.005). From the highest to the lowest knock-down rate, we have trehalose with 69.5% (63.2%-75.2%), fructose 59.9% (54.8%-64.7%), sucrose 56.9% (51.7%-61.9%) and glucose 56.3% (50.8%-61.7%). Sugar concentration also significantly influenced knockdown rate (χ2=8.74, df=1, p=0.003). Knock-down was 64.0% (60.0%–67.6%) at the lowest concentration, versus 56.2% (52.4%–59.9%) at the highest. There was no interaction between sugar concentration and the type of sugar (χ2=1.42, df=3, p=0.701), (**Fig 1B**).

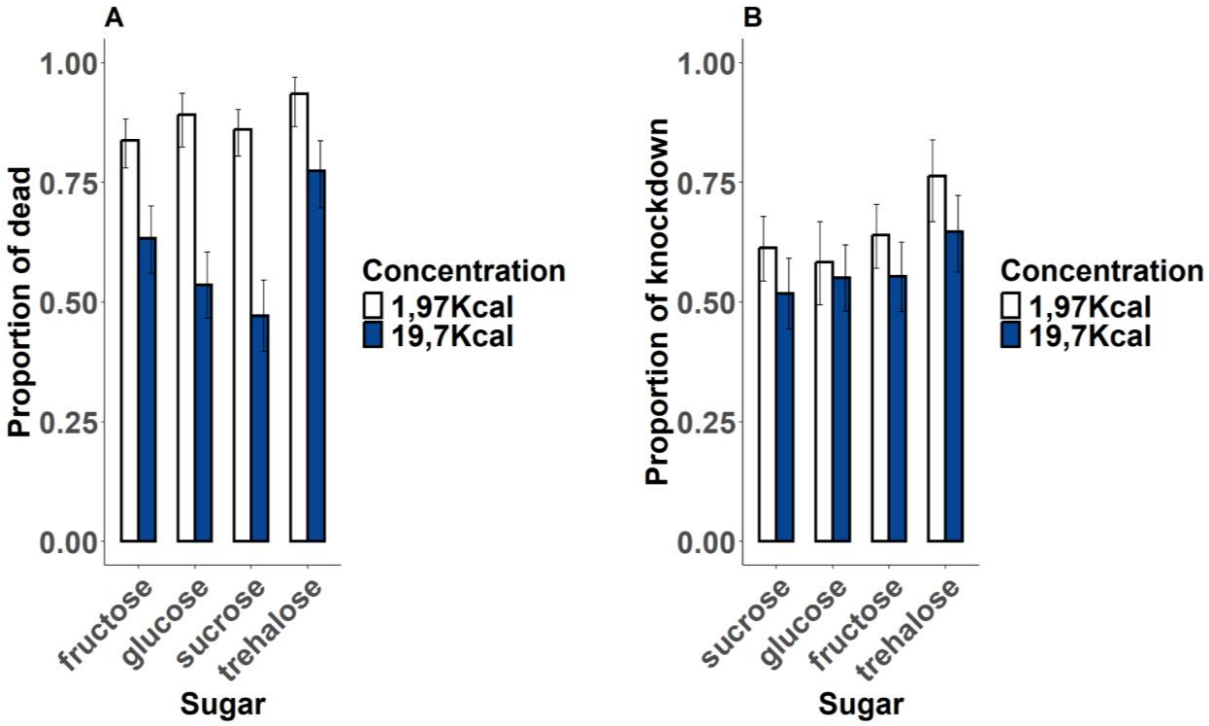

Figure 1: Effect of sugar-based diet (type and concentration) on mosquitoes' response to insecticide within 24 hours of exposure to 0.5% deltamethrin ; A) the rates of mortality ; B) the rates of knock-down. The error bars represent the 95% confidence intervals of the proportions.

Concentration of caffeine

We measured the effect of caffeine concentration on the response of 363 female mosquitoes to 0.25% deltamethrin; 22.6% died within 24 hours. The concentrations of caffeine significantly affect the mortality rate of mosquitoes (χ2=34.76;df=2; p < 0.001). The Higher the concentration of caffeine the more the mortality rate increased, 0 ppm: 11.4% (6.9%-18.2%), 50 ppm : 15.3% (9.9%-22.8%) and 200 ppm 41.0% (32.7%-49.9%), (**Fig 2A**).

62.2% of the mosquitoes were knocked-down within 1 hour of the exposure. The concentrations of caffeine significantly affect the rate of knock down of mosquitoes after exposure (χ2=97.59, df=2; p < 0.001). The Higher the concentration of caffeine the more the rate of knock down increased, 0 ppm: 37.4% (29.4%-46.2%), 50 ppm : 55.9% (46.9%-64.6%) and 200 ppm : 93.4% (87.6%-96.6%), (**Fig 2 B**).

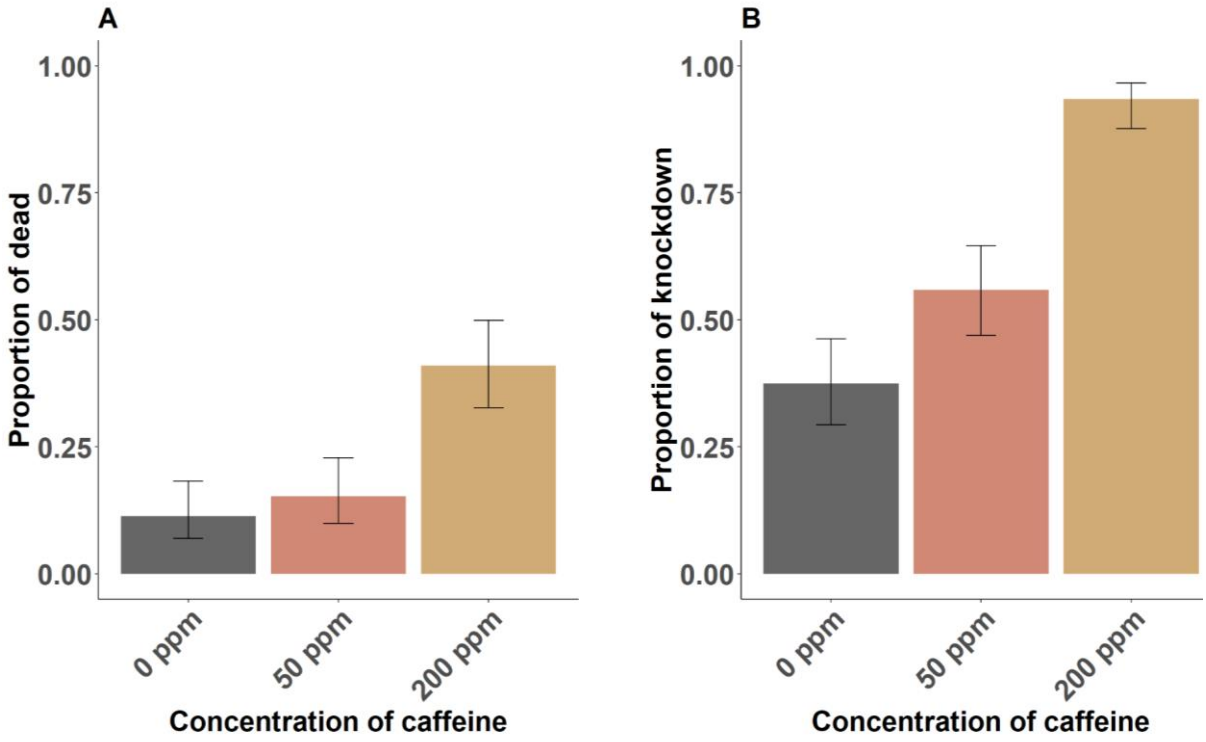

Figure 2: Effects of different concentrations of caffeine based-diet of mosquitoes on their response to insecticide within 24 hours after exposure to deltamethrin 0.25 % ; A) the rates of mortality ; B) the rates of knock-down. The error bars represent the 95% confidence intervals of the proportions.

Oxidant/Antioxidant

We measured the effect of peroxide and vitamin C on the response of 319 female mosquitoes to 0.25% deltamethrin; 26.3% died within 24 hours. Mosquitoes fed on the sugar solution supplemented with vitamin C had a significative lower mortality rate with 10.0% (5.2%–18.5%) compared to control mosquitoes (those fed without any supplement) with 26.3% (17.9% - 36.8%), (χ2=6.56; df=1; p = 0.010). Mortality was higher in mosquitoes that had received peroxide, with 38.9% of deaths (28.8%–50.1%) compared with 26.3% (17.9%–36.8%) in those that had not (the control), (χ2=11.79; df=1; p < 0.001). There was no interaction between vitamin C and hydrogen peroxide on the mortality rate, (χ2=2.03, df=1, p=0.154), (**Fig 3A**).

Mosquitoes fed exclusively on the sugar solution supplemented with vitamin C had a significative lower knockdown rate with 23.7% (15.8%-34.1%) compared to control

mosquitoes with 47.5% (36.9%-58.3%), (χ2=7.78; df=1; p = 0.005). However, the diet consisting of sugar solution with hydrogen peroxide supplementation induced a similar mortality rate of 49.35% (38.5%-603%) which is similar to control mosquitoes with 47.5% (36.9%-58.3%), (χ2=3.76; df=1; p = 0.053). There was no interaction between the diet consisting of hydrogen peroxide and vitamin C on the knockdown rate. (χ2=2.904; df=1; p = 0.088) (**Fig 3B**).

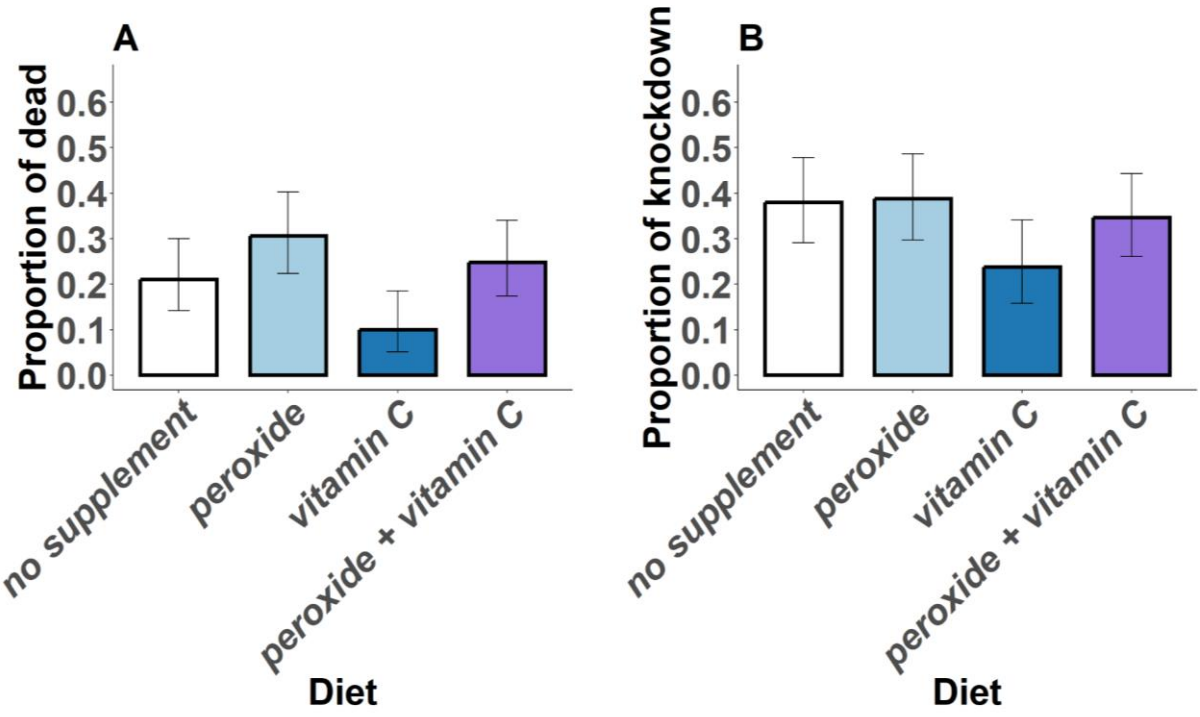

Figure 3: Effects of diets of mosquitoes based on hydrogen peroxide and/or vitamin C on their response to insecticide within 24 hours of exposure to deltamethrin 0.25 % ; A) the rates of mortality ; B) the rates of knock-down. The error bars represent the 95% confidence intervals of the proportions.

4.5. Discussion

This study demonstrates that dietary components like sugar, caffeine, vitamin C and hydrogen peroxide significantly influence the susceptibility of *Anopheles gambiae* s.l. to deltamethrin.

The concentration and type of sugar that mosquitoes feed on have strong consequences for their life-history [98] [99]. In our experiment, mosquitoes fed with the high concentration of sugar resist better than those fed with a 10-time lower concentration. Mosquitoes in this study express resistance via elevated glutathione s transferase and cytochrome P450s. Higher sugar concentrations likely enhance resistance by providing metabolic energy (ATP) needed for detoxification. This suggests that a sugar based diet supports the energy-demanding mechanisms underlying insecticide resistance.

Mosquitoes exhibited lower resistance to insecticide when fed on trehalose compared to other sugars. This aligns with recent findings, which showed that mosquitoes fed on trehalose had shorter survival times than those fed on other sugars [156]. One reason may be that the metabolism of trehalose is not efficient if glucose is lacking, which leads to a deficiency of ATP [104] [105] [106].

Vitamin C decreased mortality, while hydrogen peroxide increased it. Vitamin C decreased mosquito mortality, likely by neutralizing oxidative stress induced by insecticide exposure, while hydrogen peroxide, a reactive oxygen species (ROS), increased mortality by exacerbating oxidative damage. This pattern reflects the role of cytochrome P450 enzymes in insecticide detoxification, which generate ROS as byproducts [141]. As an antioxidant, vitamin C reduces ROS levels, improving detoxification efficiency and reinforcing insecticide resistance. In contrast, pro-oxidant exposure enhances P450 activity and ROS production, and without sufficient antioxidants to neutralize them, excessive ROS accumulation causes oxidative stress, leading to increased mosquito mortality.

Our findings are consistent with previous studies showing that oxidants influence mosquito biology. In *Anopheles gambiae,* increased ROS production in insecticide-resistant strains has been linked to reduced adult longevity [157], and hydrogen peroxide has been shown to lower emergence rates [158].

In addition to enhancing detoxification of xenobiotics, vitamin C has been shown to improve key life-history traits in mosquitoes, including lifespan, flight capacity, and mating propensity [75] [159]. While our study highlights the role of vitamin C in reinforcing insecticide resistance, other studies have reported that certain plant oils and extracts with strong

antioxidant properties act as effective larvicides [160] [161] [162] [163], and that sodium ascorbate can be toxic to adult mosquitoes [164]. These contrasting effects suggest that the impact of antioxidants depends on their chemical nature, concentration, metabolic pathways, and interactions with other compounds.

Our research shows that increasing the concentration of caffeine increases the mortality rate of mosquitoes after exposure to the insecticide. Caffeine, an alkaloid, may enhance insecticide efficacy in *Anopheles gambiae* with kdr mutations by acting as a neurotoxin and inducing oxidative stress [165] [166]. Combined with insecticide exposure, caffeine may elevate ROS beyond the mosquito's antioxidant defenses, compromising detoxification efficiency and leading to higher mortality. This mechanism is consistent with findings in *Culex quinquefasciatus*, where exposure to *Stachytarpheta jamaicensis* extracts induces elevated ROS levels, resulting in larval mortality [167]. Similarly, plant extracts reduce antioxidant enzymes in *Aedes aegypti* larvae [168] [169], and in ticks [170].
However, contrary to our findings, some larvae may exploit plant-derived metabolites such as caffeine to enhance their resistance to insecticides [171]. The caffeine concentrations used in our study, as in [77], increased mosquito fertility, illustrating that the effects of chemical compounds can differ depending on the life-history trait considered.

The fact that these phytochemicals each influenced mosquito resistance to insecticides, while our previous studies [93] showed no such effect with certain plant species, confirm the complexity of the resistance phenotype.

4.6. Conclusion

Our study demonstrates that specific phytochemicals found in floral nectar can significantly influence mosquito responses to insecticides. Nectar serves not only as an energy source but also as a reservoir of bioactive compounds capable of modulating resistance phenotypes. The type of sugar, the energy it provides, and the dietary unbalance between oxidants and antioxidants all contribute to shaping the expression of insecticide resistance in mosquitoes. This study highlights the potential of specific phytochemicals to enhance insecticide efficacy as vector control tools.

4.7. Acknowledgements

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Chapter 5: Effects of a caffeine-based diet on insecticide resistance and longevity in infected *Anopheles coluzzii*

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5.1. Abstract

Alkaloids such as caffeine can be toxic for insects. However, although mosquitoes feed on many plants with nectar containing alkaloids, their impact on the vectorial capacity of mosquitoes is not known, in particular in the face of increasing resistance to insecticides. We assessed with the mosquito *Anopheles coluzzii* how caffeine affects several measures of resistance to deltamethrin – the rate at which mosquitoes are knocked-down during exposure, the mortality within 24 hours of exposure, and the longevity following exposure – and we compared these traits for mosquitoes that were uninfected or infected by the malaria parasite *Plasmodium falciparum*. The mosquitoes were fed throughout their lives on a 10% sugar solution supplemented with 0, 50, or 200 ppm caffeine. Three or four days after emergence, they were given an infected or an uninfected blood meal. Another three days later, they were exposed to deltamethrin or to a sham and checked for knock-down during the exposure and death within the next 48 hours. We monitored the surviving mosquitoes for longevity and assessed their infection status when they died. The rate of mosquitoes knocked down by the insecticide increased with higher caffeine concentrations, but neither the infection status nor its interaction with caffeine concentration influenced the knockdown rate. Similarly, caffeine increased the mortality of insecticide-exposed mosquitoes within 48 hours after exposure. The mortality was highest if mosquitoes had fed on infected blood but harbored no parasites, and lowest if they had not fed on infected blood. The longevity, once the mosquitoes had survived the first 48 hours, was not affected by the concentration of caffeine or by any of the combination of caffeine with infection status or insecticide, but, the mosquitoes that had been exposed to the insecticide lived longer than unexposed ones, in particular if they had fed on infected blood but were not infected.

Overall, our experiment highlights that the level of resistance to an insecticide is affected by complex interactions between the mosquito's diet and infection by malaria.

Keywords: caffeine, malaria infection, insecticide resistance.

5.2. Introduction

Anopheles gambiae, the main vector of malaria in Africa, lives on average less than 10 days under natural conditions [172][173][174]. However, the malaria parasite requires approximately 11 to 14 days to complete its development into infectious stages [175] [173,176]. Indeed, this is one of the reasons why targeting adults with insecticides (either through residual indoor spraying or on insecticide-treated bed nets) to shorten the mosquitoes' life-span has become the main method of vector control [177] [178]. However, increasing mosquito resistance is reducing the effectiveness of insecticides and therefore posing a significant public health threat [179] [180] [181].

Nevertheless, insecticide-based vector control considerably decreased malaria incidence in many parts of Africa despite increasing resistance of the local malaria vectors [182] [183] [183] [184] . One of the reasons for the discrepancy between measures of resistance and their epidemiological consequences is that the impact of genes conferring resistance depends on environmental parameters like the quantity of food [117] and infection by parasites [185] including malaria [46].

An important aspect of a mosquito's diet is its nectar meal [186], and variation of the quality of nectar among plant species influences longevity [156] and resistance to insecticides [87]. Sugars are the main biochemical components of nectar [68]. This variation is partly due to the concentrations of the types of sugar in the nectar [100] [75] [187]. An additional impact of the nectar can be through its toxic compounds like alkaloids [188] [148] [189]. One alkaloid is caffeine, which occurs in the fruit and nectar of many plant species including *Coffea, Citrus, Cocoa,* tea plants [190] [191] [192] [193] [194] [195]. Although caffeine can act as an insecticide because of its neurotoxic properties [196] [86] [88] [89], not much is known about how it, on its own or in conjunction with malaria, affects insecticide-resistance.

We therefore assessed how caffeine (in concentrations commonly found in nectar) interacts with the infectious status of mosquitoes to influence their response to insecticide, measured as the rate of knock-down during exposure, the mortality within 48 hours of exposure and the longevity following exposure.

5.3. Methods

The experiment was run at the Institut de Recherches en Science de la Santé de Bobo Dioulasso (IRSS) in Burkina Faso. We used the strain 'vallée du Kou' of the mosquito *Anopheles coluzzi*, which has metabolic resistance mediated by cytochrome p450 and target-site resistance mediated by the gene kdr. The mosquitoes were maintained in a biosafety room at 27 ± 2 °C, 70 ± 5 % relative humidity and 12:12 light-dark cycle.

Experimental design

The experiment was run in three blocks, each one with the blood of a different gametocytic child as a source of malaria parasites. Within each block, we reared larvae in 40 to 50 trays containing between 250 and 300 larvae in 1 l of tap water and fed them daily with Tetramin baby fish food according to their age (day of hatching: 14 mg per tray; 1 day old: 21 mg; 2 days old: 29 mg; 3 days old: 58 mg; 4 days old: 115 mg, 5 days old or older: 216 mg). Adult males were discarded, and females were given continual access to a 10% glucose solution supplemented with 0, 50 or 200 ppm of caffeine and were given the opportunity to feed on infected or uninfected blood three or four days after emergence. (See below for the method of infection.) Each treatment was replicated in four cups of 50 to 65 mosquitoes. The females that did not take a full blood meal were discarded, leaving between 18 and 30 mosquitoes per cup). 72 hours later they were exposed to 0.25% deltamethrin or to a sham for 1 hour with the WHO bioassay kit [125]. The mosquitoes of each cup were tested together.

During the exposure to the insecticide, we counted the number of mosquitoes that were knocked down, and 48 hours after the exposure we counted the number of dead mosquitoes. The survivors were maintained for at most 30 days with continual access to their diet, and their mortality was assessed every day. Once they had died, the mosquitoes that had fed on infected blood were tested for the presence of malaria parasites with standard PCR-methods [197]; within each block 100 haphazardly selected mosquitoes that had fed on uninfected blood were also tested for malaria to ensure the absence of parasites. Infection status was classified as 'Uninfected' for those fed on an uninfected blood, 'Harbored parasite' for those fed on infected blood and have been harbored parasite and 'No harbored parasite' for those fed on infected blood but not harboring any parasites.

Experimental infection

For each block, 200 five- to 15-year-old schoolchildren were recruited in Nasso village, located 13 km from Bobo-Dioulasso, Burkina Faso. Female mosquitoes were exposed to blood samples from naturally *P. falciparum* gametocyte-infected from these children using a direct membrane feeding assay (DMFA) as described previously [78,198] [57] [198].

Briefly, thick blood smears were taken from each volunteer, air-dried, Giemsa-stained and examined by microscopy for the presence of *P. falciparum* at the IRSS laboratory in Bobo-Dioulasso. Asexual trophozoite parasite stages were counted against 200 leucocytes, while infectious gametocyte stages were counted against 1000 leukocytes. Children with asexual parasitaemia of > 1000 parasites per microlitre (estimated based on an average of 8000 leucocytes ml^{-1}) were treated in accordance with national guidelines. Asymptomatic *P. falciparum* gametocyte-positive children were recruited for the study. Blood from gametocyte isolates was collected by venipuncture in heparinized tubes. Three distinct parasite isolates (named hereafter A, B and C), with respective gametocytaemia of 56, 72 and 128 gametocytes μl^{-1} of blood, were used for the experimental infections. DMFA was performed by replacing donor plasma with an equivalent volume of AB + serum from malaria-naïve European donors. After centrifugation (Eppendorf, centrifuge 5702 R) at 37 °C at 12,000 rpm for 5 min. A horizontal line marking the upper limit of the plasma phase was drawn on the tube. The plasma was aspirated with a pipette, and AB + serum was introduced up to the marked line.

For each gametocyte carrier, half of the blood sample was transferred into 1.5 mL Eppendorf tubes and inactivated at 45 °C for 20 minutes at 900 rpm using a compact thermomixer [57]. The mosquitoes were starved of sugar solution for 24 h and then fed on infected or uninfected blood via membrane filters for 1 hour.

Data analysis

The statistical analyses were performed with R version R-4.4.2 [199]. We found the significance of the effects with the function Anova (package car) [200], using a type 3 SS if the interactions were significant and a type 2 if they were not.
We analysed the knock-down proportion and 48-hour mortality using GLMs. For mortality, we included diet, infection status, insecticide and their interactions as fixed factors. For the knock-down analysis, we replaced infection status with blood type. Since only 0.13% of mosquitoes

in the group not exposed to insecticide were knocked down, compared to 59.62% in the insecticide-exposed group, we focused solely on the mosquitoes that were exposed to insecticide. In both models, blocks (source of blood) and cups were included as random effects. In the analysis of longevity we included only the mosquitoes that had survived the first 48 hours after being exposed to the insecticide to avoid confounding impacts on longevity and on mortality. 196 mosquitoes lived longer than 30 days and were right-censored. We used a coxme model that included the diet, the infection status, the exposure to insecticide, and all interactions as fixed factors and the block (source of blood) and cups as random factors.

5.4. Results

Knockdown

Of the 1632 mosquitoes exposed to insecticide, 59.6% were knocked down within 1 hour of the exposure. The knock-down rate increased with caffeine concentration from 41.4% (95% c.i: 24.26%-60.9%) at 0 ppm, 61.4 % (41.7%-78.0%) at 50 ppm to 73.7% (53.7%-87.1%) at 200 ppm, (χ^2=38.43, df=1, p<0.001). However, neither the type of the blood ingested (χ^2=0.55, df=1, p=0.459) nor the interaction between the caffeine concentration and the type of blood ingested (χ^2=1.80, df=1, p=0.408) had an effect on the rate of knock-down **(Fig1).**

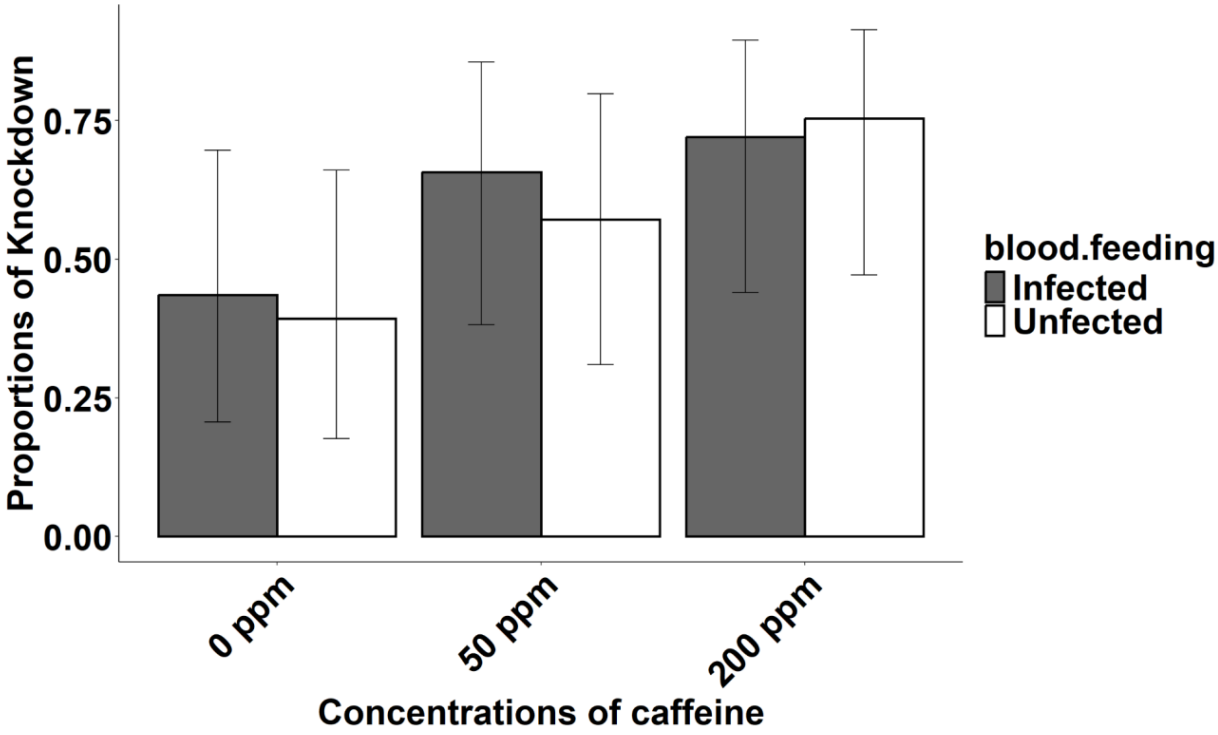

Figure 1: Proportion of mosquitoes knocked-down one hour after exposure to insecticide as a function of the concentration of caffeine and their infection status. The error bars represent the 95% confidence intervals of the proportions.

Mortality

Of the 1,559 mosquitoes exposed to insecticide, 53.2% (95% CI: 50.7%–55.6%) died within 48 hours, compared to 9.4% (95% CI: 8.0%–11.0%) of the 1,471 mosquitoes exposed to the sham. Insecticide exposure significantly increased 48-hour post-exposure mortality, (χ^2 = 260.79, df= 1,p < 0.001).

Caffeine diet alone did not influence the mortality rate, (χ^2=0.81, df=2, p=0.668). However, there was a combined effect of caffeine and insecticide exposure: Without insecticide, mortality was relatively low, ranging from 7.0% (5.1 %- 9.7%) at 200 ppm to 10.2% (7.9% - 13.1%) at 0 ppm. In the presence of insecticide, mortality increased significantly, reaching 43.6% (39.3% - 48.0%) at 0 ppm, 50.4% (46.1% - 54.6%) at 50 ppm and 64.8% (60.7% - 68.7%) at 200 ppm, (χ^2=17.76, df=2, p<0.001).

Mortality varied according to infection status. Infected mosquitoes harboring parasites had a lower mortality rate with 23.9% (21.3 - 26.7%), while infected mosquitoes that have not harbouring any parasites had a higher mortality of 47.4% (43.4 - 51.4%). Uninfected mosquitoes had an intermediate mortality of 30.8% (28.5 - 33.2%), (χ^2=17.50, df=2, p<0.001).

A significant interaction was detected between insecticide exposure and infection status. Mortality increased across all groups after insecticide exposure, and the magnitude varied: 33.8 percentage points in those infected and harboring parasite [8.5% (6.4%–11.3%) to 42.3% (37.7%–46.9%)], 54.4 in those wich infected but did not harboring parasite [14.2% (10.3%–19.2%) to 68.6%(63.7%–73.1%)], and 43.5 in uninfected mosquitoes [8.4% (6.6%–10.7%) to 51.9%(48.4%–55.5%)], (χ^2 = 7.25, df = 2, p= 0.026).

There was no significant interaction between the caffeine-based diet and infection status (χ^2=8.87, df=4, p=0.064), but a significant three-way interaction between infection status, insecticide exposure, and caffeine concentration influenced mosquito mortality (χ^2=9.85, df=4, p=0.043). At 0 ppm caffeine, mortality after insecticide exposure was highest in those which have been infected but don't harboring parasite [63.7% (54.5%-72.0%) vs. infected and harboring parasite 37.7% (30.4%-45.7%) and uninfected 37.4% (31.4%-43.9%)]. At 200 ppm, caffeine reduced mortality in non-exposed mosquitoes infected which did not harboring any parasite 2.5% (0.7%-8.6%), but mortality after exposure remained highest in this group 77.7% (69.5%-84.2%) vs. uninfected 68.5% (62.7%-73.8%) and those infected harboring parasite 47.3%(39.3%-55.3%), **(Fig 2)**.

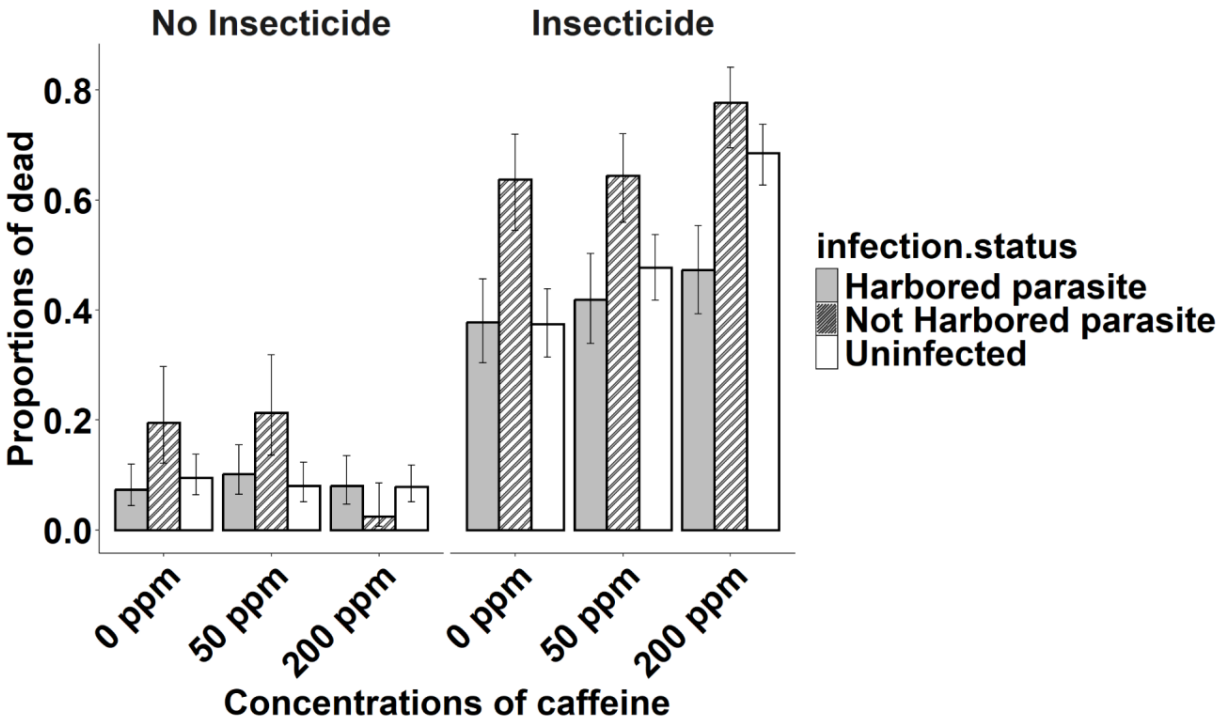

Figure 2: Mortality within 48 hours of exposure as a function of the exposure to the insecticide, concentration of caffeine and the infection status of mosquitoes. The error bars represent the 95% confidence intervals of the proportions.

Longevity

The average longevity of the 2,063 mosquitoes that survived the first 48 hours was 18.6 days. It was not significantly affected by caffeine concentration (χ2=1.46, df=2, p=0.48), nor by any of its interactions (χ2<6.4, df=2-4, p>0.17).

Longevity was about 1 day longer among insecticide-exposed mosquitoes than among unexposed ones (χ2=4.49, df=1, p =0.034). The effect of exposure depended on whether the mosquitoes were infected by malaria. In infected mosquitoes harboring parasites, the average longevity was 18.3 (17.5-19.1) days without exposure to the insecticide and 17.7 days (16.5-18.8) with exposure. In infected mosquitoes which didn't harbor parasites, the average longevity was 17.5 days (16.3-18.6) without insecticide exposure and 19.6 days (17.9-21.9) with insecticide exposure. In uninfected mosquitoes, average longevity was 18.7 days (18.0-19.3) without insecticide and 20.0 days (19.0-20.9) with insecticide exposure (interaction exposure * infection status: χ2=6.06, df=2, p=0.048), **(Fig 3).**

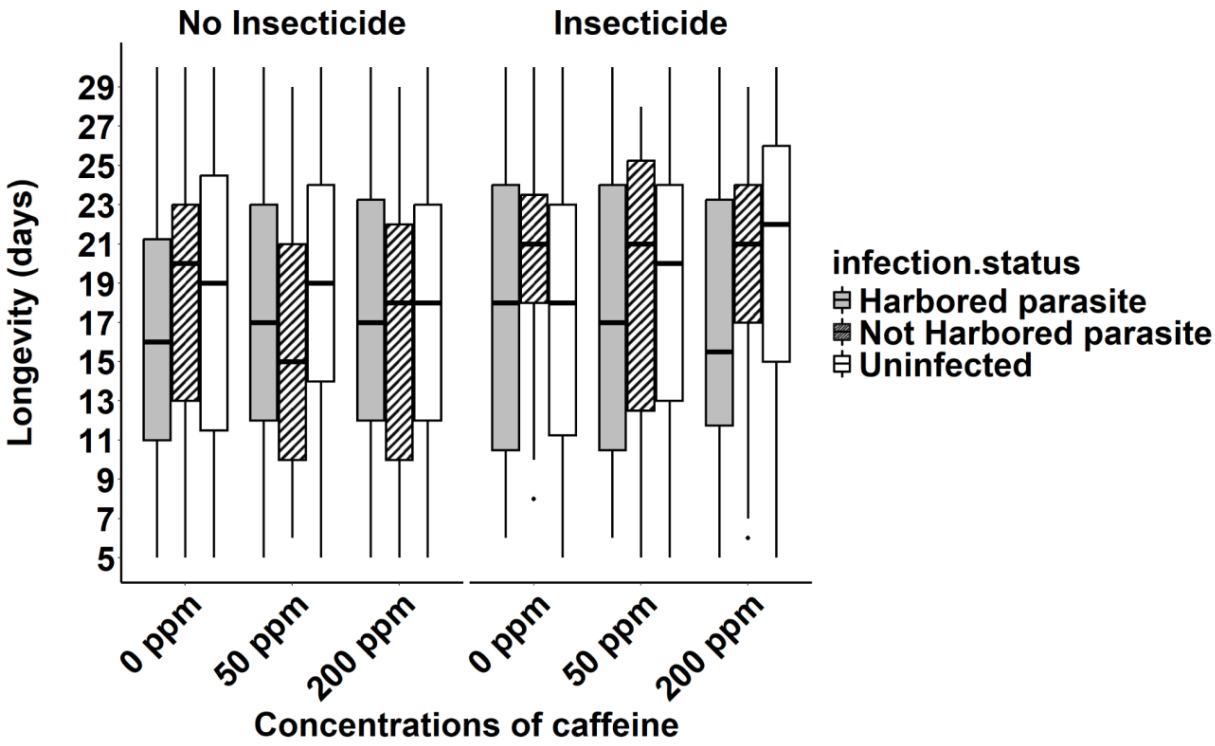

Figure 3: Longevity Starting from 48 hours after exposure) as a function of the exposure to the insecticide, Effects of different concentrations of caffeine and the infection status of mosquitoes. The rectangles represent the longevities between the 25th and the 75th percentiles, the horizontal lines within the rectangles denote the medians. The vertical lines span 1.5 times above the 75th percentiles and 1.5 times below 25th percentiles and the dots show outliers that are beyond this range.

5.5. Discussion

Our study examined how caffeine and mosquito infection status influence the phenotype of resistance and the longevity of survivors after exposure to insecticide.

Mortality increased as caffeine concentration rose in the presence of insecticide exposure.

Our work shows that increasing the concentration of caffeine increases the mortality rate of mosquitoes after exposure to the insecticide. Caffeine, an alkaloid, may enhance insecticide efficacy in *Anopheles gambiae* by acting as a neurotoxin and inducing oxidative stress [165] [166] [201]. Since exposure to the insecticide also induces ROS production via the activity of cytochrome P450. The combination of insecticide detoxification and caffeine-induced oxidative stress overloads the physiological defenses impairing detoxification enzyme efficiency and reducing resistance to insecticide, thereby increasing mortality.

Our findings align with those of Cissé-Niambélé and Koella (chapter 4), we observed increasing mortality of mosquitoes with rising caffeine concentration. Previous studies on *Aedes* larvae similarly demonstrated dose-dependent toxicity of caffeine [202] [203].

Infected mosquitoes that did not harbor any parasites and were exposed to insecticide were more susceptible to insecticide compared to other mosquitoes

The infected mosquitoes which did not harbor any parasites may have mounted an immune response against the infection, even though no parasites were ultimately detected [204] [205] [206] [207] [208]. Additionally, the immune response is often linked to an increased production of reactive oxygen species (ROS) [209] [210] [211] which may amplify the cytotoxic effects of the insecticide, thereby increasing mortality [212]. The high mortality rate could, therefore, result from the combined effects of the immune response cost and the oxidative stress induced by the insecticide toxicity.

Caffeine alone had no effect on the longevity of the survivors

The absence of an effect of caffeine on mosquito longevity was unexpected, given that caffeine is both a neurotoxic and pro-oxidant compound, and especially since it significantly affected mosquito resistance within 48 hours after insecticide exposure. Our result contrasts with several studies that have reported an effect of caffeine on various mosquito life-history traits. For instance, it has been shown to increase the locomotion of larva stage [213], and on the other hand, it would lead to a reduction of fertility in the species *Aedes albopictus* [214].

Other studies have shown that a caffeine-based diet reduces the longevity of *Aedes albopictus* at the same concentrations used in our study [77]. In addition, a reduction of longevity was observed in some flies species *Musca domestica* [215], *Drosophila prosaltans* et *D. melanogaster [216]* [217] due to their caffeine-based diet. Caffeine increases the lifespan of bees [218].

Insecticide exposure increased the general longevity of mosquitoes

The improved survival of mosquitoes exposed to insecticide can be attributed to selective sorting that favors larger individuals, which likely benefit from energy reserves accumulated during their larval stage [117] [219]. Larger mosquitoes tend to possess a higher teneral reserve, which may enhance their longevity [220].

Mosquitoes exposed to *Plasmodium falciparum* but not infected show high mortality within 48 hours of exposure to the insecticide, but survivors live longer than those in other groups.

This phenomenon could be explained by the fact that exposure to gametocytes without actual infection would trigger an immune or metabolic response, enhancing their long-term survival after the elimination of the weakest individuals.

Our study has several limitations. First, the mosquitoes used originated from a single laboratory strain known for its high level of insecticide resistance and were reared under controlled conditions. This may limit the generalizability of our findings to wild *Anopheles gambiae* populations, which may exhibit different ecological and physiological characteristics. Second, the detection of *Plasmodium* DNA in some mosquitoes does not confirm the presence of viable or developing parasites, as PCR may amplify viable or non-viable DNA. Finally, since only one insecticide (deltamethrin) was tested, the results cannot be extrapolated to other classes of insecticides, to which resistant mosquitoes might respond differently.

5.6. Conclusion

Our study shows that a caffeine-based diet increases mosquito mortality within 48 hours following insecticide exposure, but does not affect the longevity of survivors. Moreover, infection status did not interact with the caffeine diet to influence either mortality 48 hours after exposure or longevity of the survivors.

The results highlight the potential of caffeine as a complementary vector control tool, capable of enhancing mosquito susceptibility to insecticides.

This study underlines the complexity relationship between diet, the phenotype of resistance and infection status of mosquitoes.

5.7. Ethics statement

Ethical approval was given by the Centre Muraz Institutional Ethics Committee and the National Ethics Committee of Burkina Faso, N° A010-2023/ CEIRES/IRSS, 1 Avril 2023. Before we tested the children for malaria, their legal guardians provided written consent on behalf of the children. Before we obtained the blood from the three children, further informed consent was obtained from their legal guardians.

5.8. Acknowledgements

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Chapter 6: General discussion

6.1. Summary of results

The longevity of mosquitoes varies from one plant species to another, although the concentration of total sugar alone in the nectar does not explain this longevity. Additionally, the plant-based diets had no effect on mosquito resistance to insecticides. In contrast, specific phytochemicals like vitamin C, hydrogen peroxide and caffeine significantly influenced mosquito responses to insecticide. No interaction was observed between caffeine based diet and infection status in shaping either insecticide response or longevity.

Longevity of mosquitoes

Mosquitoes fed on the high concentration of sugar lived longer than those fed on a 10-time lower concentration, and mosquitoes lived longer on sucrose than on the other sugars, in particular at the low concentration. This corroborates an experiment in which mosquitoes fed on sucrose accumulated more energy reserves than those fed on glucose or fructose [100].
These findings on individual sugar diets are consistent with observations from natural nectar sources, where mosquito longevity also varies depending on the plant species consumed. The differences in longevity of the mosquitoes feeding on different plant species align with previous findings [59] [64,78] [107], in particular the long life when feeding on *T. nerifolia* [63] and the short life when feeding on *Carica papaya* [64,78].

Nectar meal size intake varied among plant species and mosquitoes survived better when they had access to the plant on which they fed more. That suggests that mosquitoes prefer some food sources over others. Such preferences can be linked to physical traits of flowers [110] [110] or to chemical emissions of the plants and nectars [61].
However, the longevity of mosquitoes was only slightly affected by the concentrations of total sugar in the sugar meals. This result suggests that nectar contains several other nutrients that largely influence mosquito longevity. This result contrasts with studies showing a positive correlation between sugar content in plant parts and mosquito survival [61].

Mosquitoes response to insecticide

Plant-based feeding did not affect mosquito resistance to insecticides, whether or not they had taken a blood meal, were old or young, or whether the time between blood meal and insecticide exposure was short or long. Indeed, while these plants provide sufficient cumulative energy for longevity, they may not be able to respond to the high energy requirements needed for rapid insecticide detoxification. The lack of difference between our plants suggests that other primary and secondary metabolites override the impact of sugar.

Dietary components like sugar, caffeine, vitamin C and hydrogen peroxide significantly influence the susceptibility of *Anopheles gambiae* s.l. to deltamethrin.
Mosquitoes fed with the high concentration of sugar resist better than those fed with a 10-time lower concentration. This result suggests that higher sugar concentrations likely enhance resistance by providing metabolic energy (ATP) needed for detoxification. The type of sugar also influences the response of mosquitoes to insecticide. In fact, mosquitoes exhibited lower resistance to insecticide when fed on trehalose compared to other sugars. This aligns with recent findings which showed that mosquitoes fed on trehalose had shorter survival times than those fed on other sugars [156]. One reason may be that the metabolism of trehalose is not efficient if glucose is lacking, which leads to a deficiency of ATP [104] [105] [106].

Vitamin C decreased mortality, while hydrogen peroxide increased it. As an antioxidant, vitamin C reduces ROS levels, improving detoxification efficiency and reinforcing insecticide resistance. In contrast, pro-oxidant exposure enhances P450 activity and ROS production, and without sufficient antioxidants to neutralize them, excessive ROS accumulation causes oxidative stress, leading to increased mosquito mortality.

Infected mosquitoes response to insecticide and their longevity

No interaction was observed between caffeine and infection status in shaping either insecticide response or mosquito longevity. However, we found that increasing the concentration of caffeine increases the mortality rate of mosquitoes after exposure to the insecticide. That can be explained by the fact that caffeine, an alkaloid, may enhance insecticide efficacy in *Anopheles gambiae* by acting as a neurotoxin and inducing oxidative stress [165] [166] [201]. Since exposure to the insecticide also induces ROS production via the activity of cytochrome P450. The combination of insecticide detoxification and caffeine-induced oxidative stress

overloads the physiological defenses impairing detoxification enzyme efficiency and reducing resistance to insecticide, thereby increasing mortality.

Infected mosquitoes that did not harbor any parasites and were exposed to insecticide were more susceptible to insecticide compared to other mosquitoes. These mosquitoes may have mounted an immune response against the infection, even though no parasites were ultimately detected [204] [205] [206]. [207] [208]. Additionally, the immune response is often linked to an increased production of reactive oxygen species (ROS) [209] [210] [211] which may amplify the cytotoxic effects of the insecticide, thereby increasing mortality [212]. The high mortality rate could, therefore, result from the combined effects of the immune response cost and the oxidative stress induced by the insecticide toxicity. Larger mosquitoes tend to possess a higher teneral reserve, which may enhance their longevity [220].

6.2. Further perspectives of research

The results of this study make a significant contribution to our understanding of the interactions between mosquitoes' diet and their longevity and, on the other hand, mosquitoes' diet and their response to insecticides. These findings open important avenues for future investigation:

- **Diet–resistance mechanism interactions**

A critical next step is to explore whether the influence of adult mosquito diet on insecticide resistance varies depending on the underlying resistance mechanism—particularly between metabolic resistance and target-site mutations. This potential interaction remains largely unexplored and could provide key insights into context-specific vulnerabilities in resistant mosquito populations.

- **Impact of diet on the Extrinsic Incubation Period (EIP)**

Another promising line of inquiry is to determine whether adult dietary composition affects the extrinsic incubation period of *Plasmodium falciparum*. While the role of larval nutrition in shaping vector competence has been documented, the effect of adult sugar source and its phytochemical profile on parasite development timing is not well understood.

- **Field-based validation**

To ensure ecological relevance, future studies should aim to validate laboratory findings under natural conditions by integrating botanical surveys, phytochemical analyses of nectar sources,

and resistance phenotyping in wild mosquito populations. Such integrative research could inform the development of novel vector control strategies that account for mosquito feeding behavior and local plant ecology.

6. 3. Conclusion

This thesis reveals that mosquito longevity and susceptibility to insecticides are not solely determined by nectar or individual sugar concentrations, but are significantly influenced by other nectar components, particularly phytochemicals like caffeine,vitamin C and hydrogen peroxide. While different plant species vary in sugar content, their nectar can have comparable effects on mosquito mortality and resistance expression, highlighting the complex role of diet in modulating resistance phenotypes. Our findings show that caffeine increases insecticide-induced mortality without affecting survivor longevity, independently of infection status. These results underscore the importance of considering mosquito diet—especially nectar phytochemicals—in resistance management strategies and support the integration of compounds like caffeine into vector control tools such as Attractive Targeted Sugar Baits (ATSBs).

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References

1. Tuteja R. Malaria - an overview: Malaria − an overview. FEBS J. 2007;274:4670–9.

2. Bruce-Chwatt LJ. Alphonse Laveran's discovery 100 years ago and today's global fight against malaria. J R Soc Med. 1981;74:531–6.

3. Ross R. On some Peculiar Pigmented Cells Found in Two Mosquitos Fed on Malarial Blood. Br Med J. 1897;2:1786–8.

4. World malaria report 2022. Geneva: World Health Organization. 2022.

5. Escalante AA, Pacheco MA. Malaria Molecular Epidemiology: An evolutionary genetics perspective. Microbiol Spectr [Internet]. 2019;7. Available from: http://dx.doi.org/10.1128/microbiolspec.AME-0010-2019

6. Guttery DS, Zeeshan M, Ferguson DJP, Holder AA, Tewari R. Division and Transmission: Malaria Parasite Development in the Mosquito. Annu Rev Microbiol. 2022;76:113–34.

7. Antony HA, Parija SC. Antimalarial drug resistance: An overview. Trop Parasitol. 2016;6:30–41.

8. Severe malaria. Trop Med Int Health. 2014;19 Suppl 1:7–131.

9. Mischlinger J, Rönnberg C, Álvarez-Martínez MJ, Bühler S, Paul M, Schlagenhauf P, et al. Imported Malaria in Countries where Malaria Is Not Endemic: a Comparison of Semi-immune and Nonimmune Travelers. Clin Microbiol Rev [Internet]. 2020;33. Available from: http://dx.doi.org/10.1128/CMR.00104-19

10. Snow RW, Gouws E, Omumbo J, Rapuoda B, Craig MH, Tanser FC, et al. Models to predict the intensity of Plasmodium falciparum transmission: applications to the burden of disease in Kenya. Trans R Soc Trop Med Hyg. 1998;92:601–6.

11. Gallup JL, Sachs JD. The economic burden of malaria. American Society of Tropical Medicine and Hygiene; 2001.

12. Andrade MV, Noronha K, Diniz BPC, Guedes G, Carvalho LR, Silva VA, et al. The economic burden of malaria: a systematic review. Malar J. 2022;21:283.

13. Goodman C, Coleman P, Mills A. Economic analysis of malaria control in sub-Saharan Africa. Geneva: Global Forum for Health Research. 2000;

14. Brooker S, Guyatt H, Omumbo J, Shretta R, Drake L, Ouma J. Situation analysis of malaria in school-aged children in Kenya – what can be done? Parasitol Today. 2000;16:183–6.

15. Shaw WR, Attardo GM, Aksoy S, Catteruccia F. A comparative analysis of reproductive biology of insect vectors of human disease. Curr Opin Insect Sci. 2015;10:142–8.

16. Koutsos AC, Blass C, Meister S, Schmidt S, MacCallum RM, Soares MB, et al. Life cycle transcriptome of the malaria mosquito Anopheles gambiae and comparison with the fruitfly Drosophila melanogaster. Proc Natl Acad Sci U S A. 2007;104:11304–9.

17. Smith RC, Vega-Rodríguez J, Jacobs-Lorena M. The Plasmodium bottleneck: malaria parasite losses in the mosquito vector. Mem Inst Oswaldo Cruz. 2014;109:644–61.

18. Smith RC, Jacobs-Lorena M. Plasmodium–Mosquito Interactions. Advances in Insect Physiology. Elsevier; 2010. p. 119–49.

19. Francischetti IMB, Sa-Nunes A, Mans BJ, Santos IM, Ribeiro JMC. The role of saliva in tick feeding. Front Biosci (Landmark Ed). 2009;14:2051–88.

20. Hopp CS, Sinnis P. The innate and adaptive response to mosquito saliva and 67 Plasmodium sporozoites in the skin. Ann N Y Acad Sci. 2015;1342:37–43.

21. Makanjuola RO, Taylor-Robinson AW. Improving accuracy of malaria diagnosis in underserved rural and remote endemic areas of SubSaharan Africa: a call to develop multiplexing rapid diagnostic tests. Scientifca. 2020;2020.

22. Borgheti-Cardoso LN, Anselmo MS, Lantero E, Lancelot A, Serrano JL, Hernández-Ainsa S. Promising nanomaterials in the fght against malaria. J Mater Chem B. 2020;8:9428–48.

23. Maier AG, Matuschewski K, Zhang M, Rug M. Plasmodium falciparum. Trends Parasitol. 2019;35:481–2.

24. Guttery DS, Holder AA, Tewari R. Sexual development in Plasmodium: lessons from functional analyses. PLoS Pathog. 2012;8:e1002404.

25. Premji ZG. Coartem: the journey to the clinic. Malar J. 2009;8 Suppl 1:S3.

26. Gachelin G, Garner P, Ferroni E, Troehler U, Chalmers I. Evaluating quinine for treating and preventing malaria. Part 1. J R Soc Med. 2017;110:31–40.

27. Talapko J, Škrlec I, Alebić T, Jukić M, Včev A. Malaria: The past and the present. Microorganisms. 2019;7:179.

28. Phyo AP, Ashley EA, Anderson TJC, Bozdech Z, Carrara VI, Sriprawat K, et al. Declining efficacy of artemisinin combination therapy against*P. falciparum*malaria on the Thai–Myanmar border (2003–2013): The role of parasite genetic factors. Clin Infect Dis. 2016;63:784–91.

29. van der Pluijm RW, Imwong M, Chau NH, Hoa NT, Thuy-Nhien NT, Thanh NV, et al. Determinants of dihydroartemisinin-piperaquine treatment failure in Plasmodium falciparum malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. Lancet Infect Dis. 2019;19:952–61.

30. Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana S-I, Yamauchi M, et al. Evidence of artemisinin-resistant malaria in Africa. N Engl J Med. 2021;385:1163–71.

31. Fikadu M, Ashenafi E. Malaria: An overview. Infect Drug Resist. 2023;16:3339–47.

32. World Health Organisation. Malaria vaccines: WHO Position Paper. 2022.

33. Tsoumani ME, Voyiatzaki C, Efstathiou A. Malaria vaccines: From the past towards the mRNA vaccine era. Vaccines (Basel) [Internet]. 2023;11. Available from: http://dx.doi.org/10.3390/vaccines11091452

34. Stanisic DI, Good MF. Malaria vaccines: Progress to date. BioDrugs. 2023;37:737–56.

35. (gmp) GMP. WHO Guidelines for malaria [Internet]. World Health Organization; 2023 [cited 2024 Nov 8]. Available from: https://www.who.int/publications/i/item/guidelines-for-malaria

36. Kouadio F-PA, Wipf NC, Nygble AS, Fodjo BK, Sadia CG, Vontas J, et al. Relationship between insecticide resistance profiles in Anopheles gambiae sensu lato and agricultural practices in Côte d'Ivoire. Parasit Vectors. 2023;16:270.

37. Gardner AM, Anderson TK, Hamer GL, Johnson DE, Varela KE, Walker ED, et al.

Terrestrial vegetation and aquatic chemistry influence larval mosquito abundance in catch basins, Chicago, USA. Parasites & Vectors. 2013;6:1–11.

38. Tusting LS, Thwing J, Sinclair D, Fillinger U, Gimnig J, Bonner KE, et al. Mosquito larval source management for controlling malaria. Cochrane Database Syst Rev. 2013;2013:CD008923.

39. World Health Organization. Toolkit for Integrated Vector Management in Sub-Saharan Africa (A). World Health Organization; 2016.

40. McCann RS, Kabaghe AN, Moraga P, Gowelo S, Mburu MM, Tizifa T, et al. The effect of community-driven larval source management and house improvement on malaria transmission when added to the standard malaria control strategies in Malawi: a cluster-randomized controlled trial. Malar J. 2021;20:232.

41. Gachelin G, Garner P, Ferroni E, Verhave JP, Opinel A. Evidence and strategies for malaria prevention and control: a historical analysis. Malar J. 2018;17:96.

42. Nosten F, Richard-Lenoble D, Danis M. A brief history of malaria. Presse Med. 2022;51:104130.

43. World Health Organization. World malaria report 2020: 20 years of global progress and challenges. World Health Organization; 2020.

44. Kleinschmidt I, Bradley J, Knox TB, Mnzava AP, Kafy HT, Mbogo C, et al. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. Lancet Infect Dis. 2018;18:640–9.

45. Oxborough RM, Chilito KCF, Tokponnon F, Messenger LA. Malaria vector control in sub-Saharan Africa: complex trade-offs to combat the growing threat of insecticide resistance. Lancet Planet Health. 2024;8:e804–12.

46. Suh PF, Elanga-Ndille E, Tchouakui M, Sandeu MM, Tagne D, Wondji C, et al. Impact of insecticide resistance on malaria vector competence: a literature review. Malaria Journal. 2023;22:1–11.

47. Sheldon BC, Verhulst S. Ecological immunology: costly parasite defences and trade-offs

in evolutionary ecology. Trends Ecol Evol. 1996;11:317–21.

48. Zhu J-H, Javid B. Tuberculosis: hey there, lonely guy! Mol Cell. 2015;57:951–2.

49. Nolan T. Control of malaria-transmitting mosquitoes using gene drives. Philos Trans R Soc Lond B Biol Sci. 2021;376:20190803.

50. Connolly JB, Burt A, Christophides G, Diabate A, Habtewold T, Hancock PA, et al. Considerations for first field trials of low-threshold gene drive for malaria vector control. Malar J. 2024;23:156.

51. Taye B, Lelisa K, Emana D, Asale A, Yewhalaw D. Seasonal Dynamics, Longevity, and Biting Activity of Anopheline Mosquitoes in Southwestern Ethiopia. J Insect Sci [Internet]. 2016;16. Available from: http://dx.doi.org/10.1093/jisesa/iev150

52. Batume C, Akol AM, Mukwaya LG, Birungi J, Kayondo JK. Life-history attributes of juvenile Anopheles gambiae s.s. in central Uganda; implications for malaria control interventions. Med Vet Entomol. 2022;36:212–22.

53. Blanco-Sierra L, Mariani S, Escartin S, Eritja R, Palmer JRB, Bartumeus F. Drivers of longevity of wild-caught Aedes albopictus populations. Parasit Vectors. 2023;16:328.

54. Posidonio APV, Oliveira LHG, Rique HL, Nunes FC. The longevity of Aedes aegypti mosquitoes is determined by carbohydrate intake. Arq Bras Med Vet Zootec. 2021;73:162–8.

55. Rezende FO, da Silva DA, Comini S, de Mendonça S, Santos E, Baldon L, et al. Dietary Influences on the Longevity and Reproductive Success of in Laboratory Studies: Sucrose vs. Honey. Insects [Internet]. 2024;15. Available from: http://dx.doi.org/10.3390/insects15120978

56. Vézilier J, Nicot A, Gandon S, Rivero A. Plasmodium infection decreases fecundity and increases survival of mosquitoes. Proc Biol Sci. 2012;279:4033–41.

57. Intervention reducing malaria parasite load in vector mosquitoes: No impact on Plasmodium falciparum extrinsic incubation period and the survival of Anopheles gambiae. PLOS Pathogens. 2023;19:e1011084.

58. Foster WA. Mosquito sugar feeding and reproductive energetics. Annu Rev Entomol. 1995;40:443–74.

59. Manda H, Gouagna LC, Nyandat E, Kabiru EW, Jackson RR, Foster WA, et al. Discriminative feeding behaviour of Anopheles gambiae s.s. on endemic plants in western Kenya. Med Vet Entomol. 2007;21:103–11.

60. Foster WA, Takken W. Substances volatiles liées au nectar et à l'homme : réponse comportementale et choix des Anopheles gambiae femelles et mâles (Diptera : Culicidae) entre l'émergence et la première alimentation. Bulletin de recherches entomologiques. 2004;94:145–57.

61. Manda H, Gouagna LC, Foster WA, Jackson RR, Beier JC, Githure JI, et al. Effect of discriminative plant-sugar feeding on the survival and fecundity of Anopheles gambiae. Malar J. 2007;6:113.

62. Muturi EJ, Allan BF, Ricci J. Influence of leaf detritus type on production and longevity of container-breeding mosquitoes. Environ Entomol. 2012;41:1062–8.

63. Hien DFDS, Dabiré KR, Roche B, Diabaté A, Yerbanga RS, Cohuet A, et al. Plant-mediated effects on mosquito capacity to transmit human malaria. PLoS Pathog. 2016;12:e1005773.

64. Nignan C, Niang A, Maïga H, Sawadogo SP, Poda BS, Gnankine O, et al. Comparison of swarming, mating performance and longevity of males Anopheles coluzzii between individuals fed with different natural fruit juices in laboratory and semi-field conditions. Malar J. 2020;19:173.

65. Stone CM, Jackson BT, Foster WA. Effects of plant-community composition on the vectorial capacity and fitness of the malaria mosquito Anopheles gambiae. Am J Trop Med Hyg. 2012;87:727–36.

66. Gary RE Jr, Foster WA. Effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector Anopheles gambiae (Diptera: Culicidae). J Med Entomol. 2001;38:22–8.

67. Heil M. Nectar: generation, regulation and ecological functions. Trends Plant Sci. 2011;16:191–200.

68. Nicolson SW. Sweet solutions: nectar chemistry and quality. Philos Trans R Soc Lond B

Biol Sci. 2022;377:20210163.

69. Foster WA. Mosquito pollination of plants: an overview of their role and an assessment of the possible contribution of disease vectors. Transgenic Res. 2024;33:297–322.

70. Nepi M, Soligo C, Nocentini D, Abate M, Guarnieri M, Cai G, et al. Amino acids and protein profile in floral nectar: Much more than a simple reward. Flora. 2012;207:475–81.

71. Boose DL. Sources of variation in floral nectar production rate in Epilobium canum (Onagraceae): implications for natural selection. Oecologia. 1997;110:493–500.

72. Parachnowitsch AL, Manson JS, Sletvold N. Evolutionary ecology of nectar. Ann Bot. 2019;123:247–61.

73. Ignell R, Okawa S, Englund J-E, Hill SR. Assessment of diet choice by the yellow fever mosquito Aedes aegypti. Physiol Entomol. 2010;35:274–86.

74. Airs PM, Kudrna KE, Bartholomay LC. Impact of sugar composition on meal distribution, longevity, and insecticide toxicity in Aedes aegypti. Acta Trop. 2019;191:221–7.

75. Phasomkusolsil S, Pantuwatana K, Tawong J, Khongtak W, Kertmanee Y, Monkanna N, et al. Sugar and multivitamin diet effects on the longevity and mating capacity of laboratory-reared male anopheline mosquitoes. J Am Mosq Control Assoc. 2017;33:175–83.

76. Nascimento DAS, Trindade FTT, Silva A de AE. Dietary supplementation with vitamins and minerals improves larvae and adult rearing conditions of Anopheles darlingi (Diptera: Culicidae). J Med Entomol. 2021;58:71–8.

77. Njoroge TM, Calla B, Berenbaum MR, Stone CM. Specific phytochemicals in floral nectar up-regulate genes involved in longevity regulation and xenobiotic metabolism, extending mosquito life span. Ecol Evol. 2021;11:8363–80.

78. Hien DFDS, Paré PSL, Cooper A, Koama BK, Guissou E, Yaméogo KB, et al. Contrasting effects of the alkaloid ricinine on the capacity of Anopheles gambiae and Anopheles coluzzii to transmit Plasmodium falciparum. Parasit Vectors. 2021;14:479.

79. Moyo P, Mugumbate G, Eloff JN, Louw AI, Maharaj VJ, Birkholtz L-M. Natural products: A potential source of malaria transmission blocking drugs? Pharmaceuticals (Basel).

2020;13:251.

80. Rivero A, Magaud A, Nicot A, Vézilier J. Energetic cost of insecticide resistance in Culex pipiens mosquitoes. J Med Entomol. 2011;48:694–700.

81. Alout H, Dabiré RK, Djogbénou LS, Abate L, Corbel V, Chandre F, et al. Interactive cost of Plasmodium infection and insecticide resistance in the malaria vector Anopheles gambiae. Sci Rep. 2016;6:29755.

82. Alout H, Yameogo B, Djogbénou LS, Chandre F, Dabiré RK, Corbel V, et al. Interplay between Plasmodium infection and resistance to insecticides in vector mosquitoes. J Infect Dis. 2014;210:1464–70.

83. Agyekum TP, Arko-Mensah J, Botwe PK, Hogarh JN, Issah I, Dadzie SK, et al. Relationship between temperature and Anopheles gambiae sensu lato mosquitoes' susceptibility to pyrethroids and expression of metabolic enzymes. Parasit Vectors. 2022;15:163.

84. Mseti JJ, Maasayi MS, Lugenge AG, Mpelepele AB, Kibondo UA, Tenywa FC, et al. Temperature, mosquito feeding status and mosquito density influence the measured bio-efficacy of insecticide-treated nets in cone assays. Parasit Vectors. 2024;17:159.

85. Hodjati MH, Curtis CF. Effects of permethrin at different temperatures on pyrethroid-resistant and susceptible strains of Anopheles. Med Vet Entomol. 1999;13:415–22.

86. Norris EJ, Bloomquist JR. Recording central neurophysiological output from mosquito larvae for neuropharmacological and insecticide resistance studies. J Insect Physiol. 2021;135:104319.

87. Paré PSL, Hien DFDS, Bayili K, Yerbanga RS, Cohuet A, Carrasco D, et al. Natural plant diet impacts phenotypic expression of pyrethroid resistance in Anopheles mosquitoes. Sci Rep. 2022;12:21431.

88. Nathanson JA. Caffeine and related methylxanthines: possible naturally occurring pesticides. Science. 1984;226:184–7.

89. Snel J, Lorist MM. Effects of caffeine on sleep and cognition. Prog Brain Res. 2011;190:105–17.

90. Foster WA, Takken W. Nectar-related vs. human-related volatiles: behavioural response and choice by female and male Anopheles gambiae (Diptera: Culicidae) between emergence and first feeding. Bull Entomol Res. 2004;94:145–57.

91. Carvajal-Lago L, Ruiz-López MJ, Figuerola J, Martínez-de la Puente J. Implications of diet on mosquito life history traits and pathogen transmission. Environ Res. 2021;195:110893.

92. Fodjo BK, Koudou BG, Tia E, Saric J, N'dri PB, Zoh MG, et al. Insecticides Resistance Status of in Areas of Varying Agrochemical Use in Côte D'Ivoire. Biomed Res Int. 2018;2018:2874160.

93. Cissé KN, Koella JC, Koudou GB. Effects of the diet of the mosquito Anopheles gambiae s.l. on its resistance to an insecticide. 2025 [cited 2025 Jun 10]; Available from: https://www.researchsquare.com/article/rs-5966237/latest.pdf

94. Kosaka A, Aida M, Katsumoto Y. Reconsidering the activation entropy for anomerization of glucose and mannose in water studied by NMR spectroscopy. J Mol Struct. 2015;1093:195–200.

95. Roslund MU, Tähtinen P, Niemitz M, Sjöholm R. Complete assignments of the 1H and 13C chemical shifts and JH,H coupling constants in NMR spectra of d-glucopyranose and all d-glucopyranosyl-d-glucopyranosides. Carbohydr Res. 2008;343:101–12.

96. Barclay T, Ginic-Markovic M, Johnston MR, Cooper P, Petrovsky N. Observation of the keto tautomer of D-fructose in D(2)O using (1)H NMR spectroscopy. Carbohydr Res. 2012;347:136–41.

97. Peach DAH. Sugar Detection in Mosquitoes via Anthrone Tests. Cold Spring Harb Protoc. 2024;2024:108301.

98. Gu W, Müller G, Schlein Y, Novak RJ, Beier JC. Natural plant sugar sources of Anopheles mosquitoes strongly impact malaria transmission potential. PLoS One. 2011;6:e15996.

99. Upshur IF, Fehlman M, Parikh V, Vinauger C, Lahondère C. Sugar feeding by invasive mosquito species on ornamental and wild plants. Sci Rep. 2023;13:22121.

100. Kessler S, Vlimant M, Guerin PM. Sugar-sensitive neurone responses and sugar feeding preferences influence lifespan and biting behaviours of the Afrotropical malaria mosquito,

Anopheles gambiae. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2015;201:317–29.

101. Becker A, Schlöder P, Steele JE, Wegener G. The regulation of trehalose metabolism in insects. Experientia. 1996;52:433–9.

102. Kaushik JK, Bhat R. Why is trehalose an exceptional protein stabilizer? An analysis of the thermal stability of proteins in the presence of the compatible osmolyte trehalose. J Biol Chem. 2003;278:26458–65.

103. Liu K, Dong Y, Huang Y, Rasgon JL, Agre P. Impact of trehalose transporter knockdown on Anopheles gambiae stress adaptation and susceptibility to Plasmodium falciparum infection. Proc Natl Acad Sci U S A. 2013;110:17504–9.

104. Wyatt GR, Kale GF. The chemistry of insect hemolymph. II. Trehalose and other carbohydrates. J Gen Physiol. 1957;40:833–47.

105. Becker A, Schlöder P, Wegener G. La régulation du métabolisme du tréhalose chez les insectes. Experientia. 1996;52:433–9.

106. Tellis MB, Kotkar HM, Joshi RS. Regulation of trehalose metabolism in insects: from genes to the metabolite window. Glycobiology. 2023;33:262–73.

107. Gary RE Jr, Foster WA. Anopheles gambiae feeding and survival on honeydew and extra-floral nectar of peridomestic plants. Med Vet Entomol. 2004;18:102–7.

108. Li P, Chen B, Dong Y, Xu S, Chen K. Effect of temperature and supplementary nutrition on the development, longevity and oviposition ofConopomorpha sinensis(Lepidoptera: Gracillariidae). Fla Entomol. 2013;96:338–43.

109. Vrzal EM, Allan SA, Hahn DA. Amino acids in nectar enhance longevity of female Culex quinquefasciatus mosquitoes. J Insect Physiol. 2010;56:1659–64.

110. van Rijn PCJ, Wäckers FL. Nectar accessibility determines fitness, flower choice and abundance of hoverflies that provide natural pest control. J Appl Ecol. 2016;53:925–33.

111. Gouagna LC, Kerampran R, Lebon C, Brengues C, Toty C, Wilkinson DA, et al. Sugar-source preference, sugar intake and relative nutritional benefits in Anopheles arabiensis males.

Acta Trop. 2014;132 Suppl:S70–9.

112. Martinez-Ibarra JA, Rodriguez MH, Arredondo-Jimenez JI, Yuval B. Influence of plant abundance on nectar feeding by Aedes aegypti (Diptera: Culicidae) in southern Mexico. J Med Entomol. 1997;34:589–93.

113. Müller G, Schlein Y. Nectar and honeydew feeding of Phlebotomus papatasi in a focus of Leishmania major in Neot Hakikar oasis. J Vector Ecol. 2004;29:154–8.

114. Edi CAV, Koudou BG, Bellai L, Adja AM, Chouaibou M, Bonfoh B, et al. Long-term trends in Anopheles gambiae insecticide resistance in Côte d'Ivoire. Parasit Vectors. 2014;7:500.

115. World Health Organization. World malaria report 2023. World Health Organization; 2023.

116. Ibrahim EA, Wamalwa M, Odindi J, Tonnang HEZ. Insights and challenges of insecticide resistance modelling in malaria vectors: a review. Parasit Vectors. 2024;17:174.

117. Kulma K, Saddler A, Koella JC. Effects of age and larval nutrition on phenotypic expression of insecticide-resistance in Anopheles mosquitoes. PLoS One. 2013;8:e58322.

118. Glunt KD, Oliver SV, Hunt RH, Paaijmans KP. The impact of temperature on insecticide toxicity against the malaria vectors Anopheles arabiensis and Anopheles funestus. Malar J. 2018;17:131.

119. Oliver SV, Lyons CL, Brooke BD. The effect of blood feeding on insecticide resistance intensity and adult longevity in the major malaria vector Anopheles funestus (Diptera: Culicidae). Sci Rep. 2022;12:3877.

120. Oliver SV, Brooke BD. The Role of Oxidative Stress in the Longevity and Insecticide Resistance Phenotype of the Major Malaria Vectors Anopheles arabiensis and Anopheles funestus. PLoS One. 2016;11:e0151049.

121. Champion CJ, Xu J. Redox state affects fecundity and insecticide susceptibility in Anopheles gambiae. Sci Rep. 2018;8:13054.

122. Adhikari K, Khanikor B. Gradual reduction of susceptibility and enhanced detoxifying

enzyme activities of laboratory-reared Aedes aegypti under exposure of temephos for 28 generations. Toxicol Rep. 2021;8:1883–91.

123. Hernandez EP, Anisuzzaman, Alim MA, Kawada H, Kwofie KD, Ladzekpo D, et al. Ambivalent roles of oxidative stress in triangular relationships among arthropod vectors, pathogens and hosts. Antioxidants (Basel). 2022;11:1254.

124. Website [Internet]. Available from: : https://doi.org/10.1101/2025.03.02.641044;

125. World Health Organization. Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. Genève, Switzerland: World Health Organization; 2022.

126. Website [Internet]. Available from: R Core Team (2024) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.R-project.org/

127. Website [Internet]. Available from: https://www.john-fox.ca/Companion/

128. Machani MG, Ochomo E, Sang D, Bonizzoni M, Zhou G, Githeko AK, et al. Influence of blood meal and age of mosquitoes on susceptibility to pyrethroids in Anopheles gambiae from Western Kenya. Malar J. 2019;18:112.

129. Gangaram S, Naidoo Y, Dewir YH, El-Hendawy S. Phytochemicals and biological activities of Barleria (Acanthaceae). Plants. 2021;11:82.

130. Chalcoff VR, Aizen MA, Galetto L. Nectar concentration and composition of 26 species from the temperate forest of South America. Ann Bot. 2006;97:413–21.

131. Rodríguez MM, Bisset JA, Fernández D. Determination in vivo of the role of esterase and glutathione transferase enzymes in pyrethroid resistance of Aedes aegypti (Diptera: Culicidae). Rev Cubana Med Trop. 2007;59:209–12.

132. Martinez-Sobrido L, Toral FA. New Advances on Zika Virus Research. MDPI; 2019.

133. Oliveira JHM, Gonçalves RLS, Lara FA, Dias FA, Gandara ACP, Menna-Barreto RFS, et al. Blood meal-derived heme decreases ROS levels in the midgut of Aedes aegypti and allows proliferation of intestinal microbiota. PLoS Pathog. 2011;7:e1001320.

134. Briegel H, Lea AO. Relationship between protein and proteolytic activity in the midgut

of mosquitoes. J Insect Physiol. 1975;21:1597–604.

135. Esquivel CJ, Cassone BJ, Piermarini PM. A de novo transcriptome of the Malpighian tubules in non-blood-fed and blood-fed Asian tiger mosquitoes Aedes albopictus: insights into diuresis, detoxification, and blood meal processing. PeerJ. 2016;4:e1784.

136. Chouaibou MS, Chabi J, Bingham GV, Knox TB, 'dri N, Kesse L. Augmentation de la sensibilité aux insecticides avec le vieillissement des moustiques sauvages Anopheles gambiae de Côte d'Ivoire. BMC Infect Dis. 2012;12.

137. Lines JD, Nassor NS. DDT resistance in Anopheles gambiae declines with mosquito age. Med Vet Entomol. 1991;5:261–5.

138. Mbepera S, Nkwengulila G, Peter R, Mausa EA, Mahande AM, Coetzee M. L'influence de l'âge sur la sensibilité aux insecticides d' Anopheles arabiensis pendant les saisons sèches et pluvieuses dans les périmètres d'irrigation du riz du nord de la Tanzanie. Malar J. 2017;16.

139. Hodjati MH, Curtis CF. Évaluation de l'effet de l'âge du moustique et de l'exposition antérieure à l'insecticide sur la tolérance aux pyréthroïdes chez les moustiques Anopheles (Diptera : Culicidae). Bull Entomol Res. 1999;89:329–37.

140. Jones CM, Sanou A, Guelbeogo WM, Sagnon N, Johnson PC, Ranson H. Le vieillissement restaure partiellement l'efficacité de la lutte antivectorielle contre le paludisme dans les populations d' Anopheles gambiae sl résistantes aux insecticides au Burkina Faso. Malar J. 2012;11.

141. Murataliev MB, Guzov VM, Walker FA, Feyereisen R. P450 reductase and cytochrome b5 interactions with cytochrome P450: effects on house fly CYP6A1 catalysis. Insect Biochem Mol Biol. 2008;38:1008–15.

142. Zhu YC, Caren J, Reddy GVP, Li W, Yao J. Effect of age on insecticide susceptibility and enzymatic activities of three detoxification enzymes and one invertase in honey bee workers (Apis mellifera). Comp Biochem Physiol C Toxicol Pharmacol. 2020;238:108844.

143. Durak R, Dampc J, Kula-Maximenko M, Mołoń M, Durak T. Changes in antioxidative, oxidoreductive and detoxification enzymes during development of aphids and temperature increase. Antioxidants (Basel). 2021;10:1181.

144. Saeaue L, Morales NP, Komalamisra N, Morales Vargas RE. Antioxidative systems defense against oxidative stress induced by blood meal in Aedes aegypti. Southeast Asian J Trop Med Public Health. 2011;42:542–9.

145. Oliveira JHM, Talyuli OAC, Goncalves RLS, Paiva-Silva GO, Sorgine MHF, Alvarenga PH, et al. Catalase protects Aedes aegypti from oxidative stress and increases midgut infection prevalence of Dengue but not Zika. PLoS Negl Trop Dis. 2017;11:e0005525.

146. Stevenson PC, Nicolson SW, Wright GA. Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. Funct Ecol. 2017;31:65–75.

147. Website [Internet]. Available from: Southwick, E. E. (1984). Photosynthate allocation to floral nectar: A neglected energy investment. Ecology, 65(6), 1775–1779. https://doi.org/10.2307/1937773

148. Nicolson SW, Nepi M, Pacini E. Nectaries and Nectar. Springer Science & Business Media; 2007.

149. del Río LA, Puppo A. Reactive Oxygen Species in Plant Signaling. Springer Science & Business Media; 2009.

150. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxford University Press; 2015.

151. Smirnoff N, Wheeler GL. Ascorbic acid in plants: biosynthesis and function. Crit Rev Biochem Mol Biol. 2000;35:291–314.

152. Zhang Y. Ascorbic Acid in Plants: Biosynthesis, Regulation and Enhancement. Springer Science & Business Media; 2012.

153. Website [Internet]. Available from: Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: Keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology, 49, 249–279. https://doi.org/10.1146/annurev.arplant.49.1.249

154. Website [Internet]. Available from: R Core Team (2024) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.R-project.org/

155. Website [Internet]. Available from: https://www.john-fox.ca/Companion/

156. Cissé KN, Koella JC, Koudou GB. Effects of nectar and sugar on the longevity of the mosquito Anopheles gambiae s.l [Internet]. bioRxiv. 2025 [cited 2025 Jun 11]. p. 2025.03.02.641044. Available from: https://www.biorxiv.org/content/10.1101/2025.03.02.641044v1.abstract

157. Otali D, Novak RJ, Wan W, Bu S, Moellering DR, De Luca M. Increased production of mitochondrial reactive oxygen species and reduced adult life span in an insecticide-resistant strain of Anopheles gambiae. Bull Entomol Res. 2014;104:323–33.

158. Antonio-Nkondjio C, Youmsi-Goupeyou M, Kopya E, Tene-Fossog B, Njiokou F, Costantini C, et al. Exposure to disinfectants (soap or hydrogen peroxide) increases tolerance to permethrin in Anopheles gambiae populations from the city of Yaoundé, Cameroon. Malar J. 2014;13:296.

159. Barbosa AL, Gois GC, Dos Santos VB, Pinto AT de M, de Castro Andrade BP, de Souza LB, et al. Effects of different diets on adults: improving rearing techniques for sterile insect technique. Bull Entomol Res. 2023;113:748–55.

160. John R, Sabu KR, Manilal A. Chemical Composition, Antioxidant, and Mosquito Larvicidal Activity of Essential Oils from Jacq. J Exp Pharmacol. 2022;14:195–204.

161. Mahendran G, Vimolmangkang S. Chemical compositions, antioxidant, antimicrobial, and mosquito larvicidal activity of Ocimum americanum L. and Ocimum basilicum L. leaf essential oils. BMC Complement Med Ther. 2023;23:390.

162. Yadav R, Preet S. Comparative assessment of green and chemically synthesized glutathione capped silver nanoparticles for antioxidant, mosquito larvicidal and eco-toxicological activities. Sci Rep. 2023;13:8152.

163. Udappusamy V, Mohan H, Thinagaran R. Lantana camara L. essential oil mediated nano-emulsion formulation for biocontrol application: anti-mosquitocidal, anti-microbial and antioxidant assay. Arch Microbiol. 2022;204:388.

164. McDermott EG, Morris EK, Garver LS. Sodium Ascorbate as a Potential Toxicant in Attractive Sugar Baits for Control of Adult Mosquitoes (Diptera: Culicidae) and Sand Flies (Diptera: Psychodidae). J Med Entomol. 2019;56:1359–67.

165. Alasalvar C, Shahidi F, Ho C-T. Dietary Supplements with Antioxidant Activity: Understanding Mechanisms and Potential Health Benefits. Royal Society of Chemistry; 2023.

166. Kaczmarczyk-Sedlak I, Folwarczna J, Sedlak L, Zych M, Wojnar W, Szumińska I, et al. Effect of caffeine on biomarkers of oxidative stress in lenses of rats with streptozotocin-induced diabetes. Arch Med Sci. 2019;15:1073–80.

167. Rajan V, Puthur R. Activation of the Oxidative Stress in Culex quinquefasciatus by the Augmented Production of Reactive Oxygen Species (ROS) in response to Stachytarpheta jamaicensis Exposure. J Commun Dis. 2021;53:43–51.

168. Koodalingam A, Deepalakshmi R, Ammu M, Rajalakshmi A. Effects of NeemAzal on marker enzymes and hemocyte phagocytic activity of larvae and pupae of the vector mosquito Aedes aegypti. J Asia Pac Entomol. 2014;17:175–81.

169. Thanigaivel A, Senthil-Nathan S, Vasantha-Srinivasan P, Edwin E-S, Ponsankar A, Selin-Rani S, et al. Chemicals isolated from *Justicia adhatoda* Linn reduce fitness of the mosquito, *Aedes aegypti* L. Arch Insect Biochem Physiol [Internet]. 2017;94. Available from: http://dx.doi.org/10.1002/arch.21384

170. Agwunobi DO, Pei T, Yang J, Wang X, Lv L, Shen R, et al. Expression profiles of glutathione S-transferases genes in semi-engorged Haemaphysalis longicornis (Acari: Ixodidae) exposed to Cymbopogon citratus essential oil. Syst Appl Acarol. 2020;25:918–30.

171. Hafeez M, Liu S, Jan S, Shi L, Fernández-Grandon GM, Gulzar A, et al. Knock-Down of Gossypol-Inducing Cytochrome P450 Genes Reduced Deltamethrin Sensitivity in (Hübner). Int J Mol Sci [Internet]. 2019;20. Available from: http://dx.doi.org/10.3390/ijms20092248

172. Lambert B, North A, Godfray C. A meta-analysis of longevity estimates of mosquito vectors of disease [Internet]. bioRxiv. 2022. Available from: http://biorxiv.org/lookup/doi/10.1101/2022.05.30.494059

173. Matthews J, Bethel A, Osei G. An overview of malarial Anopheles mosquito survival estimates in relation to methodology. Parasit Vectors. 2020;13:233.

174. Epopa PS, Millogo AA, Collins CM, North A, Tripet F, Benedict MQ, et al. The use of sequential mark-release-recapture experiments to estimate population size, survival and

dispersal of male mosquitoes of the Anopheles gambiae complex in Bana, a west African humid savannah village. Parasit Vectors. 2017;10:376.

175. Dvorin JD, Goldberg DE. Egress Across the Parasite Life Cycle. Annu Rev Microbiol. 2022;76:67–90.

176. Cook PE, Hugo LE, Iturbe-Ormaetxe I, Williams CR, Chenoweth SF, Ritchie SA, et al. The use of transcriptional profiles to predict adult mosquito age under field conditions. Proc Natl Acad Sci U S A. 2006;103:18060–5.

177. Alhassan Y, Dwomoh D, Amuasi SA, Nonvignon J, Bonful H, Tetteh M, et al. Impact of insecticide-treated nets and indoor residual spraying on self-reported malaria prevalence among women of reproductive age in Ghana: implication for malaria control and elimination. Malaria Journal. 2022;21:1–17.

178. Lindsay SW, Thomas MB, Kleinschmidt I. Threats to the effectiveness of insecticide-treated bednets for malaria control: thinking beyond insecticide resistance. Lancet Glob Health. 2021;9:e1325–31.

179. Maxmen A. Malaria surge feared [Internet]. Nature Publishing Group UK. 2012 [cited 2025 Mar 4]. Available from: http://dx.doi.org/10.1038/485293a

180. Ashley EA, Pyae Phyo A, Woodrow CJ. Malaria. Lancet. 2018;391:1608–21.

181. World Health Organization. Global Malaria Programme operational strategy 2024-2030. World Health Organization; 2024.

182. Henry MC, Assi SB, Rogier C, Dossou-Yovo J, Chandre F, Guillet P, et al. Protective efficacy of lambda-cyhalothrin treated nets in Anopheles gambiae pyrethroid resistance areas of Côte d'Ivoire. The American journal of tropical medicine and hygiene [Internet]. 2005 [cited 2025 May 7];73. Available from: https://pubmed.ncbi.nlm.nih.gov/16282294/

183. Lindblade KA, Mwandama D, Mzilahowa T, Steinhardt L, Gimnig J, Shah M, et al. A cohort study of the effectiveness of insecticide-treated bed nets to prevent malaria in an area of moderate pyrethroid resistance, Malawi. Malaria Journal. 2015;14:1–15.

184. Alout H, Labbé P, Chandre F, Cohuet A. Malaria Vector Control Still Matters despite Insecticide Resistance. Trends Parasitol. 2017;33:610–8.

185. Koella JC, Saddler A, Karacs TPS. Blocking the evolution of insecticide-resistant malaria vectors with a microsporidian. Evol Appl. 2012;5:283–92.

186. Herreros-Moya E, Sinka M, Harris AF, Entwistle J, Martin AC, Willis KJ. The food of life: which nectar do mosquitoes feed on?-An evidence-based meta-analysis. Environ Entomol [Internet]. 2025; Available from: http://dx.doi.org/10.1093/ee/nvaf009

187. Aiman M, Kassim NFA, Jong Z-W, Webb CE. EFFECTS OF SUGAR CONCENTRATION ON FECUNDITY, BITING BEHAVIOR AND SURVIVABILITY OF FEMALE AEDES (STEGOMYIA) ALBOPICTUS (SKUSE). Southeast Asian J Trop Med Public Health. 2016;47:1160–6.

188. Adler LS. The ecological significance of toxic nectar. Oikos. 2000;91:409–20.

189. Carter C, Thornburg RW, Nepi M. New Perspectives on the Biology of Nectaries and Nectars. Frontiers Media SA; 2019.

190. Caffeine in Citrus flowers. Phytochemistry. 1999;52:19–23.

191. Wright GA, Baker DD, Palmer MJ, Stabler D, Mustard JA, Power EF, et al. Caffeine in floral nectar enhances a pollinator's memory of reward. Science. 2013;339:1202–4.

192. Marchi IL, Palottini F, Farina WM. Combined secondary compounds naturally found in nectars enhance honeybee cognition and survival. J Exp Biol [Internet]. 2021;224. Available from: http://dx.doi.org/10.1242/jeb.239616

193. Somorin O. Spectrophotometric determination of caffeine in Nigerian kola nuts. J Food Sci. 1973;38:911–2.

194. Zhu B, Chen L-B, Lu M, Zhang J, Han J, Deng W-W, et al. Caffeine Content and Related Gene Expression: Novel Insight into Caffeine Metabolism in Camellia Plants Containing Low, Normal, and High Caffeine Concentrations. Journal of Agricultural and Food Chemistry [Internet]. 2019 [cited 2025 Mar 4]; Available from: https://pubs.acs.org/doi/abs/10.1021/acs.jafc.9b00240

195. Association mapping of caffeine content with TCS1 in tea plant and its related species. Plant Physiology and Biochemistry. 2016;105:251–9.

196. Ko BS, Ahn SH, Noh DO, Hong K-B, Han SH, Suh HJ. Effect of explosion-puffed coffee on locomotor activity and behavioral patterns in Drosophila melanogaster. Food Res Int. 2017;100:252–60.

197. Morassin B, Fabre R, Berry A, Magnaval JF. One year's experience with the polymerase chain reaction as a routine method for the diagnosis of imported malaria. Am J Trop Med Hyg. 2002;66:503–8.

198. Ouattara SB, Hien DFDS, Nao ET, Paré PSL, Guissou E, Cohuet A, et al. A simple, field-applicable method to increase the infectivity of wild isolates of Plasmodium falciparum to mosquito vectors. Malaria Journal. 2024;23:1–9.

199. Website [Internet]. Available from: R Core Team (2024) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.R-project.org/

200. Website [Internet]. Available from: https://www.john-fox.ca/Companion/

201. Gülçin I. In vitro prooxidant effect of caffeine. J Enzyme Inhib Med Chem. 2008;23:149–52.

202. Miranda FR, Fernandes KM, Bernardes RC, Martins GF. Biological, histological and immunohistochemical studies on the toxicity of spent coffee grounds and caffeine on the larvae of Aedes aegypti (Diptera: Culicidae). Environ Pollut. 2021;271:116307.

203. Guirado MM, Bicudo HEMC. Attractiveness of bioinsecticides caffeine and used coffee grounds in the choice of oviposition site by Aedes aegypti (Diptera: Culicidae). Int J Mosq Res. 2016;3:47–51.

204. Reynolds RA, Kwon H, Smith RC. 20-Hydroxyecdysone Primes Innate Immune Responses That Limit Bacterial and Malarial Parasite Survival in Anopheles gambiae. mSphere [Internet]. 2020;5. Available from: http://dx.doi.org/10.1128/mSphere.00983-19

205. Mosquito immunity against Plasmodium. Insect Biochemistry and Molecular Biology. 2005;35:677–89.

206. Dimopoulos G. Insect immunity and its implication in mosquito-malaria interactions. Cell Microbiol. 2003;5:3–14.

207. Kumar S, Christophides GK, Cantera R, Charles B, Han YS, Meister S, et al. The role of reactive oxygen species on Plasmodium melanotic encapsulation in Anopheles gambiae. Proceedings of the National Academy of Sciences. 2003;100:14139–44.

208. Oliveira JHM, Gonçalves RLS, Oliveira GA, Oliveira PL, Oliveira MF, Barillas-Mury C. Energy metabolism affects susceptibility of Anopheles gambiae mosquitoes to Plasmodium infection. Insect Biochem Mol Biol. 2011;41:349–55.

209. Molina-Cruz A, DeJong RJ, Charles B, Gupta L, Kumar S, Jaramillo-Gutierrez G, et al. Reactive oxygen species modulate Anopheles gambiae immunity against bacteria and Plasmodium. J Biol Chem. 2008;283:3217–23.

210. Surachetpong W, Pakpour N, Cheung KW, Luckhart S. Reactive oxygen species-dependent cell signaling regulates the mosquito immune response to Plasmodium falciparum. Antioxid Redox Signal. 2011;14:943–55.

211. Bahia AC, Oliveira JHM, Kubota MS, Araújo HRC, Lima JBP, Ríos-Velásquez CM, et al. The role of reactive oxygen species in Anopheles aquasalis response to Plasmodium vivax infection. PLoS One. 2013;8:e57014.

212. Mangum LC, Borazjani A, Stokes JV, Matthews AT, Lee JH, Chambers JE, et al. Organochlorine insecticides induce NADPH oxidase-dependent reactive oxygen species in human monocytic cells via phospholipase A2/arachidonic acid. Chem Res Toxicol. 2015;28:570–84.

213. Ishay JS, Paniry VA. Effects of caffeine and various xanthines on hornets and bees. Psychopharmacology . 1979;65:299–309.

214. Abernathy HA, Boyce RM, Reiskind MH. Exploring the effects of caffeine on Aedes albopictus (Diptera: Culicidae) survival and fecundity. J Med Entomol. 2023;60:837–41.

215. Srinivasan A, Kesavan PC. Effect of caffeine on longevity and reproduction of the housefly. Toxicol Lett. 1979;3:101–5.

216. Itoyama MM, Bicudo HE, Manzato AJ. The development of resistance to caffeine in Drosophila prosaltans: productivity and longevity after ten generations of treatment. Cytobios. 1998;96:81–93.

217. Suh HJ, Shin B, Han S-H, Woo MJ, Hong K-B. Behavioral changes and survival in Drosophila melanogaster: Effects of ascorbic acid, taurine, and caffeine. Biol Pharm Bull. 2017;40:1873–82.

218. Bernklau E, Bjostad L, Hogeboom A, Carlisle A, H S A. Dietary phytochemicals, honey bee longevity and pathogen tolerance. Insects. 2019;10:14.

219. Barreaux AMG, Barreaux P, Thievent K, Koella JC. Larval environment influences vector competence of the malaria mosquito Anopheles gambiae. MalariaWorld J. 2016;7:8.

220. Metabolic relationship between female body size, reserves, and fecundity of Aedes aegypti. Journal of Insect Physiology. 1990;36:165–72.