Role of natural nectar sources on malaria transmission and contribution of vectors to ecosystem services (PALUNEC)





Domonbabele François de Sales HIEN

14 / 12 / 2022

PALUNEC Project

PALUNEC is a project associated at l'Institut de Recherche et de Développement (IRD)

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Correspondant IRD: **Dr Thierry LEFEVRE**, researcher at IRD and CNRS

Membre: **Pr Olivier GNANKINE**, Research Senior Teacher in entomology at Joseph KI-ZERBO University

JEAI PALUNEC

2019-2022

Institut de Recherche pour le Développement

Membre: **Dr Rakiswendé Serge YERBANGA**, researcher in pharmacognosy-parasitology and biochemistry at IRSS and Instech

Laurrainne Prisca PARE, PhD Student on tne project

PALUNEC Project: Context 1/3

Project motivation ???

Current means of control => Phenomena of resistance

Few studies on sweet feeding of mosquito vectors

Exploring this long-neglected part of **vector bio-ecology**

PALUNEC project: Context 2/3

- Frequent ingestion of **nectar** by both sexes of mosquitoes of all ages (Foster 1995)

- **Nectar** => energy required for flight. Improves survival and fecundity in female mosquitoes (Manda et al. 2007a)

- Females able to locate plant **nectar** sources and show preferences among different plant species (Manda et al. 2007b)

- **Nectars** can influence malaria transmission (Hien et al. 2016)






PALUNEC project: Objectives 1/1

General objective:

Role of natural nectar sources on malaria transmission and contribution of vectors to ecosystem services

Specific objectives:

1. To explore the effect of a wide range of natural sugar-producing plant species on the competence and survival of *Anopheles gambiae* s.l.

2. a. To study the sugar feeding behaviour of *Anopheles gambiae* s.l. : trophic preference

2. b. To determine the sugar plant susceptibility effect of *Anopheles gambiae* s.l. to insecticides

3. To develop new Attractive Transmission-blocking Sugar Baits (ATBS)

4. To assess the role of mosquitoes in the pollination of flowering plants

Competence and survival 1/11

Screening of flowers/fruits: 34 plant species

Plumeria alba

Albizia lebbeck

Bignonia corymbosa

Centaurea aspera

Crotalaria retusa

Hyptis suaveolens

Lantana camara

Duranta erecta

Spartium junceum

Urena lobata

Bougainvillea glabra

Cassia occidentalis

Cassia alata

Senna corymbosa

Plumbago auriculata

Azadirachta indica

Saba senegalensis

Galeopsis segetum

Canna indica

Tradescantia pallida-purpurea

Antigonon leptopus

Barleria prionitis

Caesalpinia pulcherrima

Ixora coccinea

Jatropha integerrima

Tecoma stans

Tridax procumbens

Onobrychis viciifolia

Combretum indicum

Cassia siamea

Cassia sieberiana

Anogeissus leiocarpa

Sclerocarya birrea

Saba senegalensis

Vitex doniana

Competence and survival 2/11

Screening of flowers/fruits: 34 plant species

Methodology: Collecting and making flower bouquets

Fresh flowers collection

Removal of leaves from the collected flowers on aluminium foil

Washing the flowers and wrapping them with moistened paper towels and aluminium foil

Solution control:
5% glucose

Species: *An. coluzzii*

Survival: 1-7 days

Test antrhone (Van Handel, 1972)

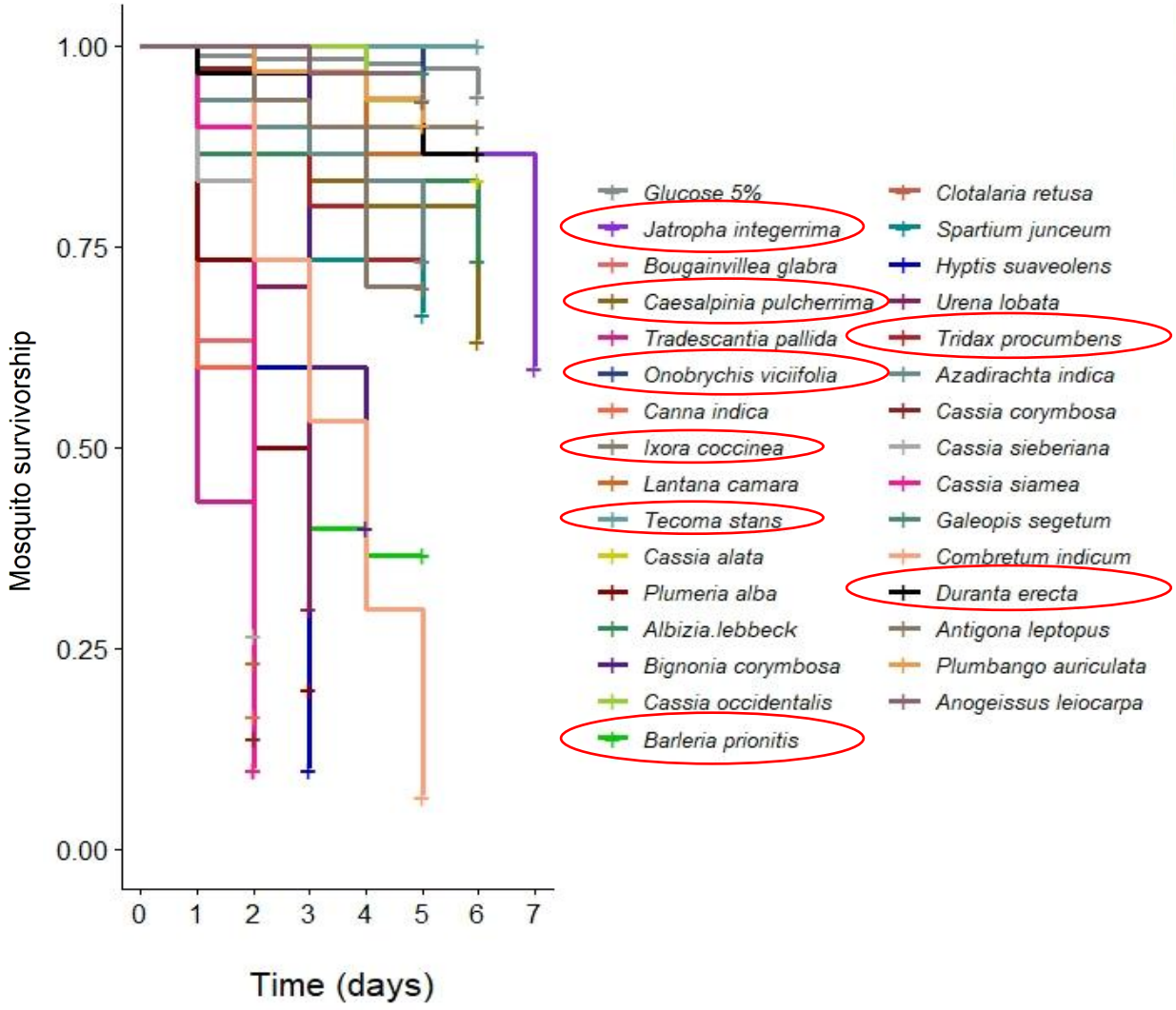
Flowers in the cages

Flower bouquets in plastic cups

Competence and survival 3/11

Screening of flowers/fruits

Results



Barleria prionitis

Caesalpinia pulcherrima

Duranta erecta

Ixora coccinea

Jatropha integerrima

Onobrychis viciifolia

Tecoma stans

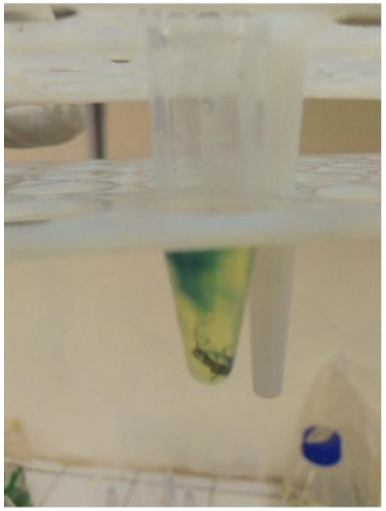
Tridax procumbens

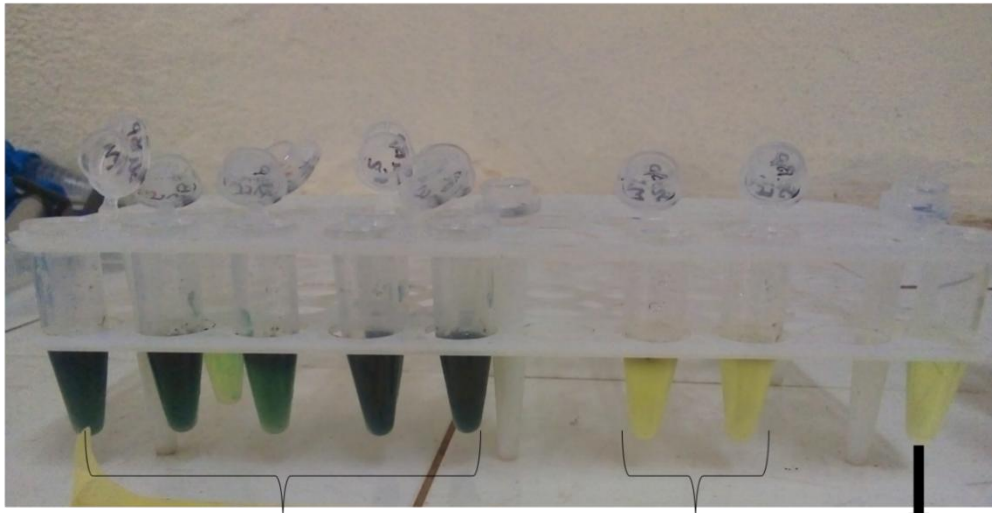
Competence and survival 4/11

Screening of flowers/fruits

Methodology: Anthrone test (Van Handel, 1972)

- Individual mosquitoes crushed in 0.5 mL of anthrone solution.
- Incubation: 60 min at room temperature
- The lemon-yellow anthrone solution reacted with fructose to give light green, blue or dark blue colours depending on the amount of fructose ingested by mosquitoes





Competence and survival 5/11

Anthrone test

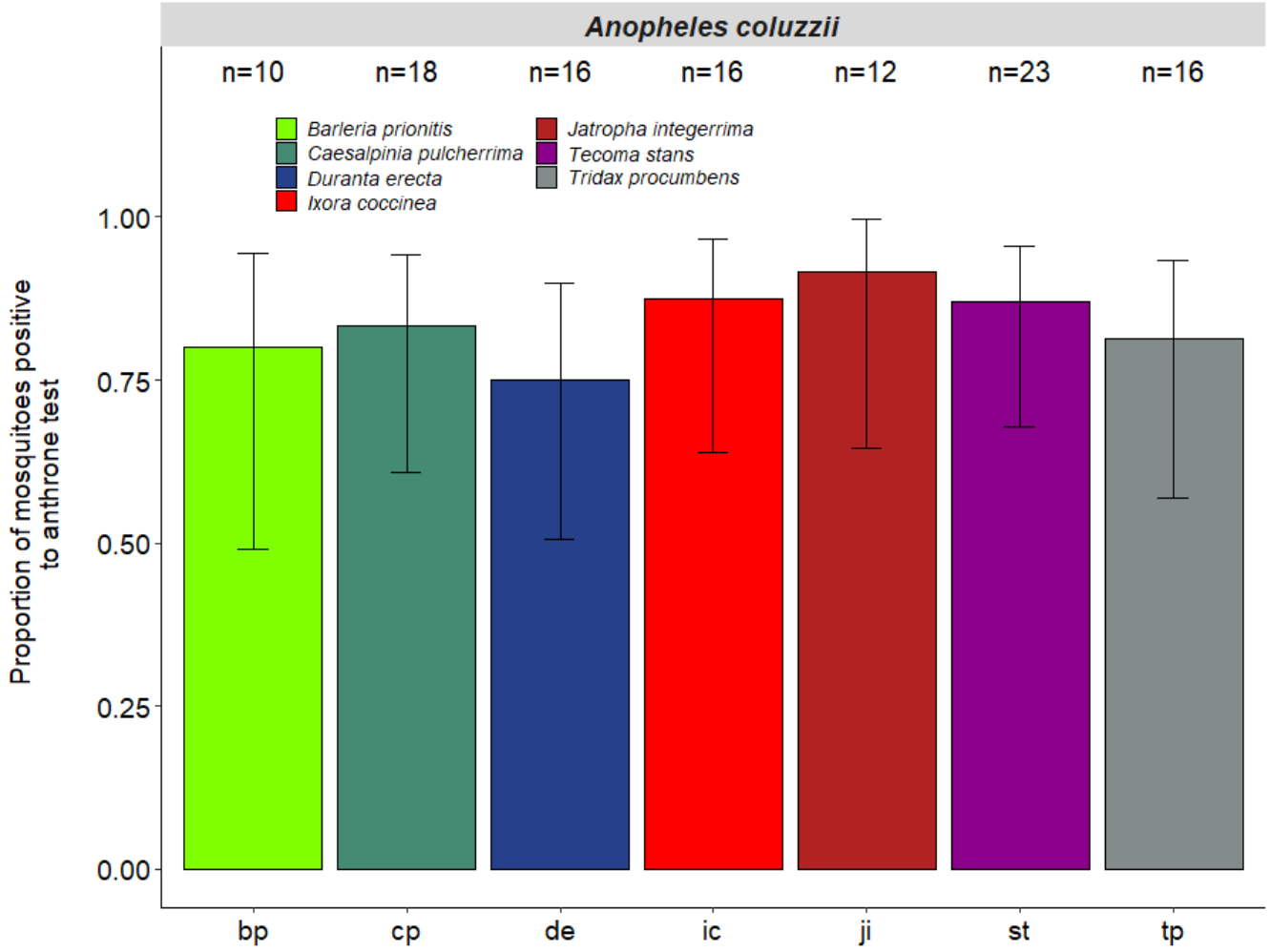


Figure 1: Proportion of mosquitoes positive to anthrone test

Competence and survival 6/11

Methodology: Experimental infection

Female (3-5 days old) of *An. gambiae s.l.* fed with blood containing *P. falciparum* gametocytes through direct membrane feeding system

Infected females cages according to types of sugar diet

5% glucose

B. prionitis

C. pulcherrima

D. erecta

I. coccinea

J. integerrima

O. viciifolia

T. stans

T. procumbens

Competence and survival 7/11

Methodology: Dissection

Competence

Oocyst prevalence 7DPI

The proportion of mosquitoes exposed to infectious blood and that had at least one oocyst in their midgut

Oocyst intensity 7DPI

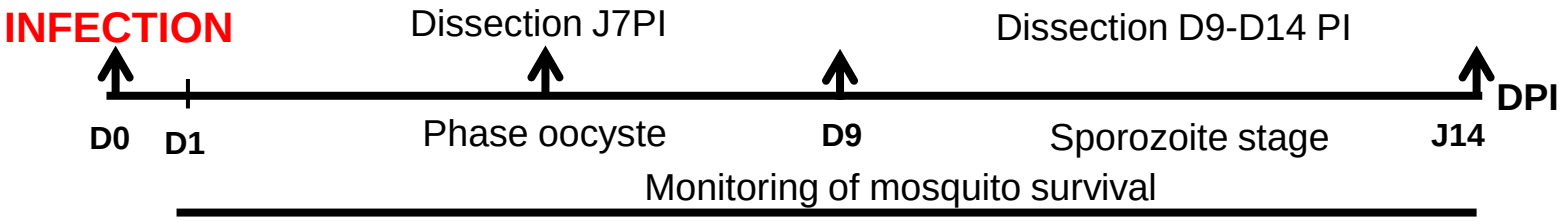
The average number of oocysts in the midgut of infected mosquitoes

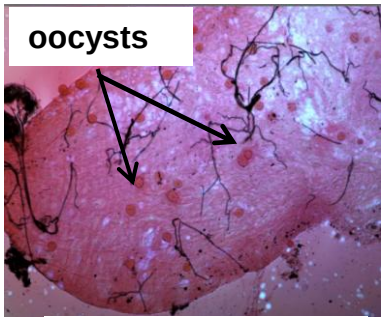
Index sporozoique

Proportion of sporozoites 9-14DPI

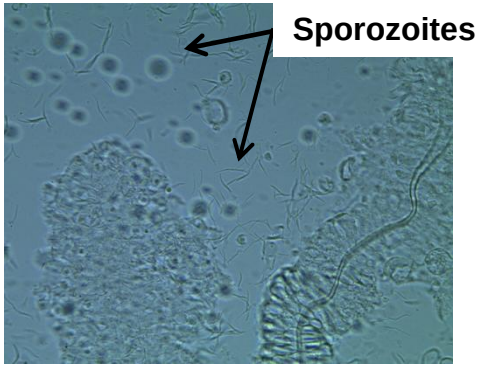
Survival

Survival 1-14DPI





midgut positive to oocysts



Glands positive to sporozoites

Competence and survival 8/11

Results: Prevalence and intensity of oocysts at 7DPI

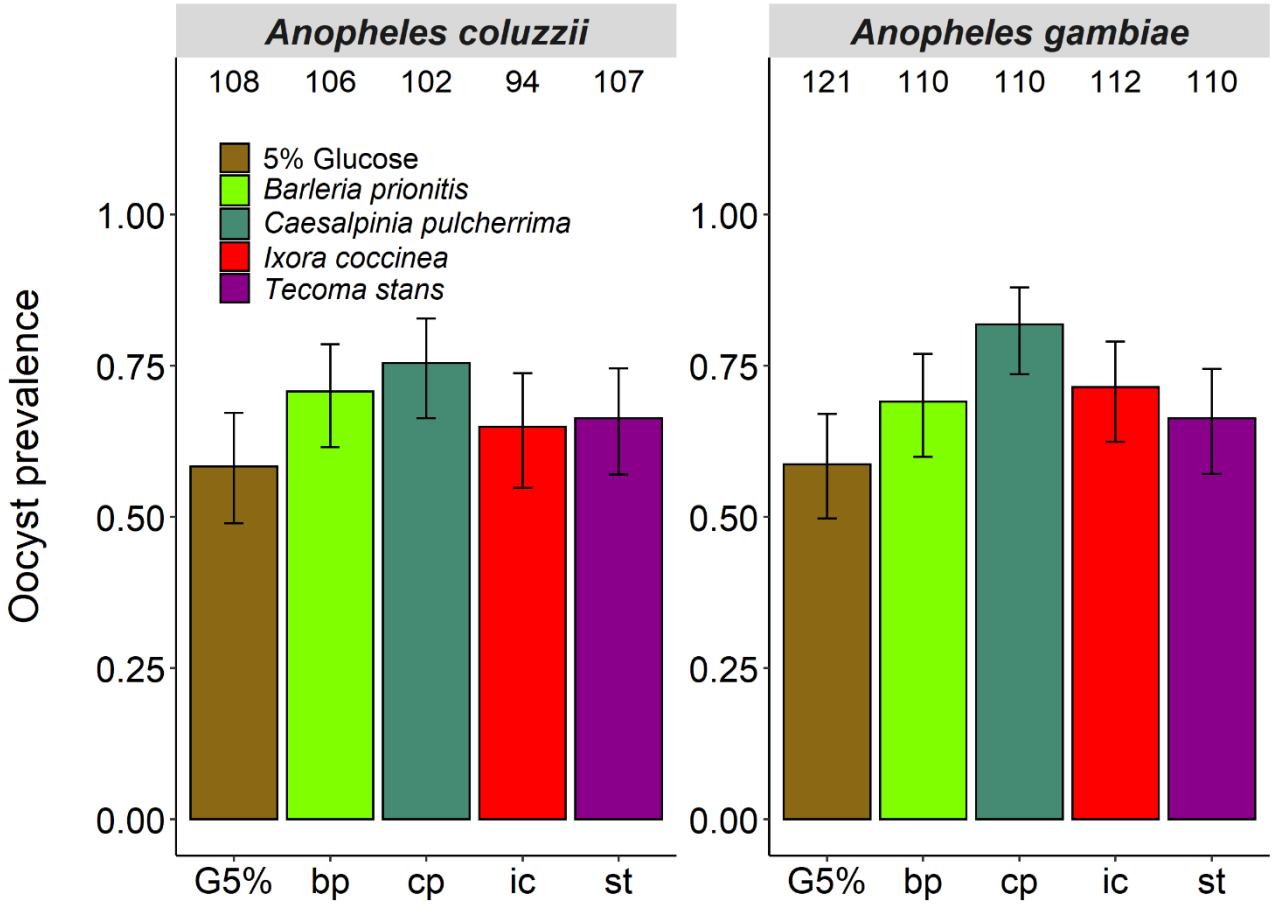


Figure 2: Oocyst prevalence

Treatment effect: LRT $X^2_4 = 44.81$; P< 0.001

Species effect: LRT $X^2_1 = 3.18$; P = 0.07

Treatment*species: LRT $X^2_4 = 5.73$, P = 0,22

G5% = 52%
cp = 73%
bp : 62%

***An. coluzzii :* 60%**
***An. gambiae :* 63%**

Competence and survival 9/11

Results: Prevalence and intensity of oocysts at 7DPI

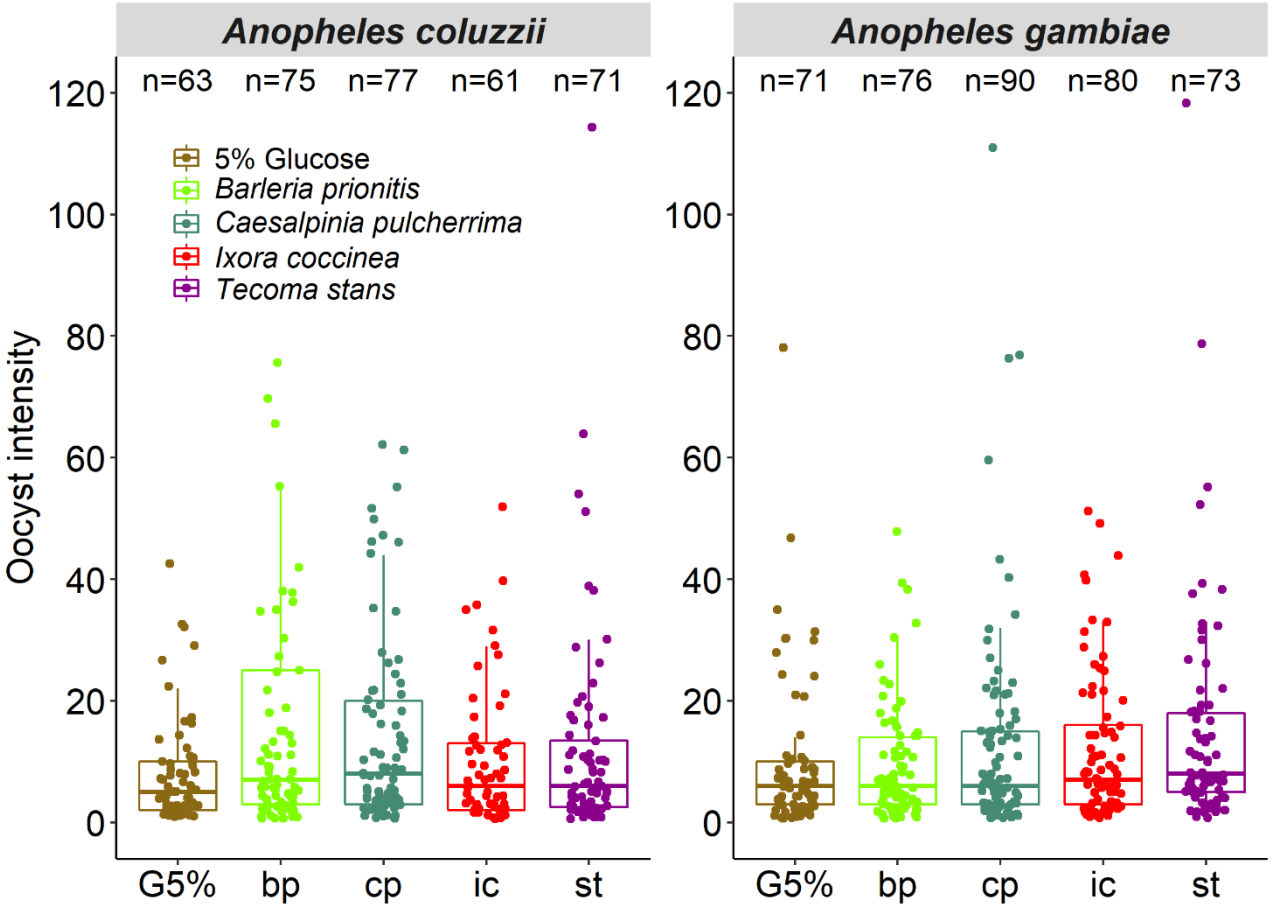


Figure 3: Oocyst intensity

Treatment effect: LRT $X^2_4 = 24.37$, P< 0.001

Species effect: LRT $X^2_1 = 15.94$; P< 0.001

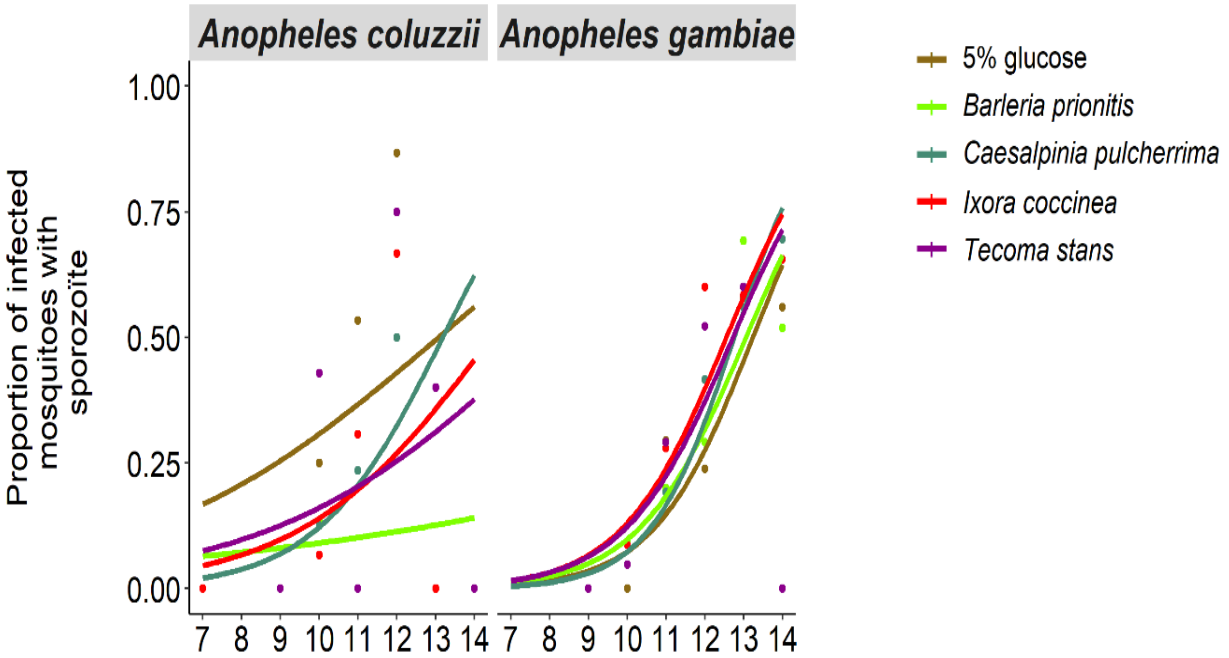
Treatment*species: LRT $X^2_4 = 26.38$, P< 0.001

G5% = 8.05 < bp=12.65
cp=10.85 < bp = 12.65
ic = 8.75 < bp = 12.65
ic = 8.75 < st = 10.48

***An. coluzzii :* 11.85**
***An. gambiae :* 9.19**

Competence and survival 10/11

Results: Temporal dynamics of the appearance of sporozoites



An. coluzzii

Treatment effect: LRT X^2_4= 12.11 , P= 0.01

bp = 6% < G5% = 24%

An. gambiae

Treatment effect: LRT X^2_4= 9.45 , P= 0.03

G5% = 19%

bp = 25%

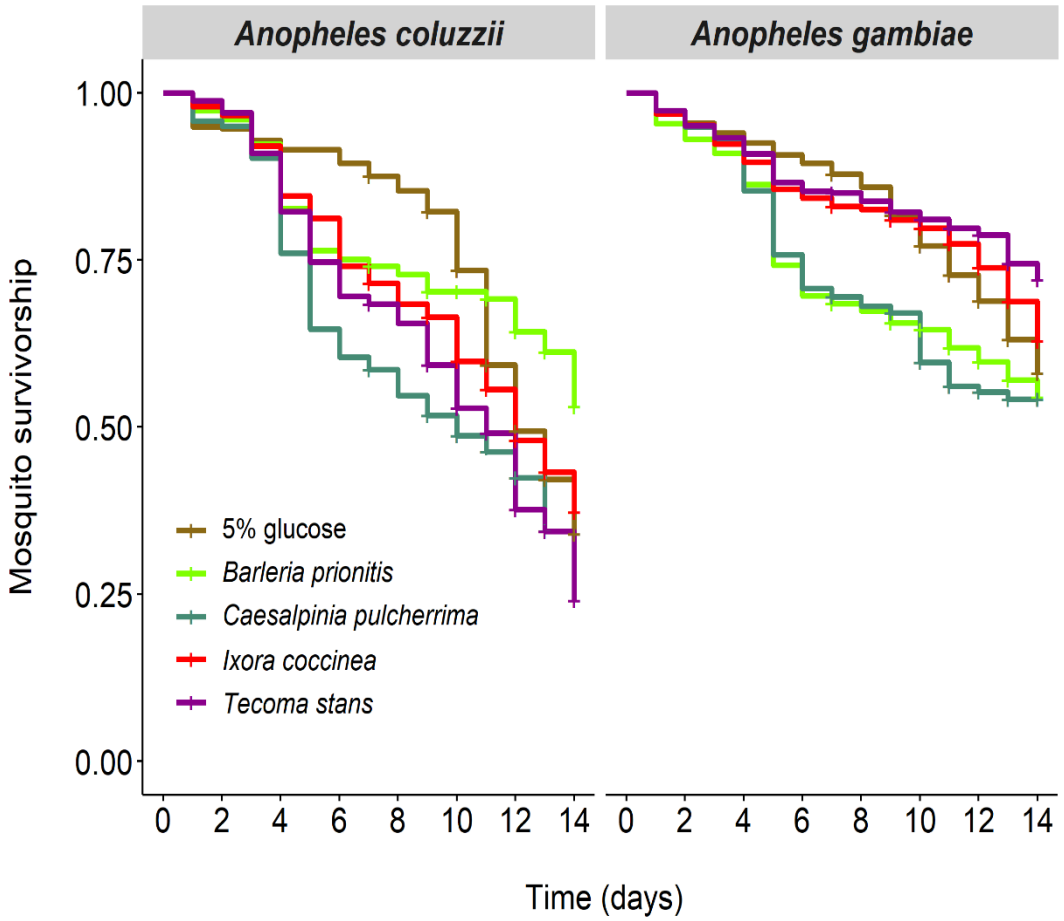
cp= 26%

ic = 30%

st = 24%

Competence and survival 11/11

Results: Survival post-infection



Treatment effect : LRT X^2_4= 69.95 , P< 0.001

Species effect : LRT X^2_1= 89.93 , P< 0.001

Treatment* species: LRT X^2_4= 50.10 , P< 0.001

Trophic preference 1/3

Methodology: Sugar diet selection

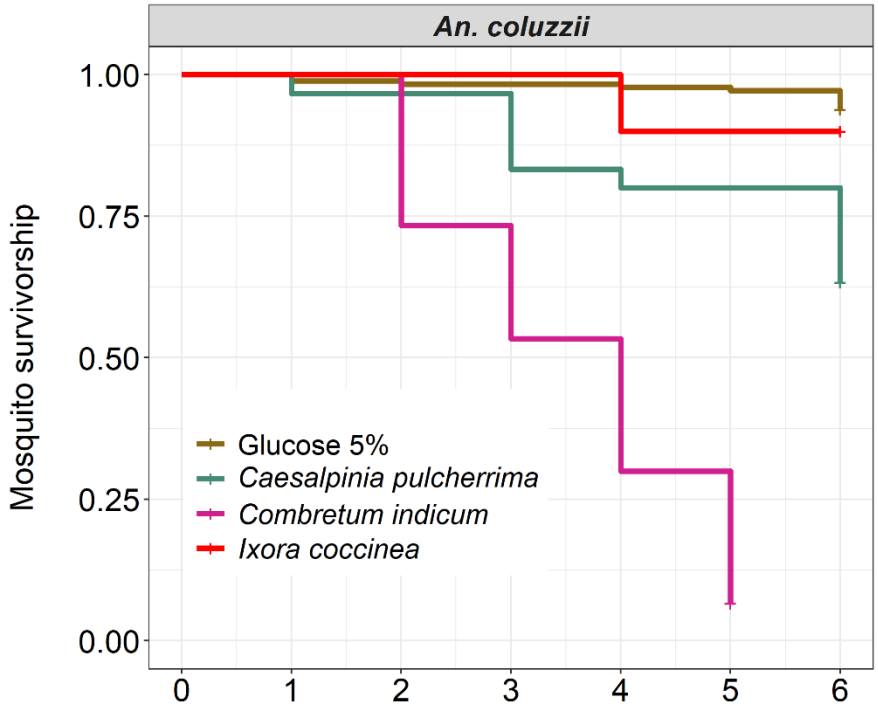
Caesalpinia pulcherrima

Combretu indicum

Ixora coccinea

5% glucose

Water



%mortality

G5% = 6 ± 0.14

ic = 10% ± 0.34

cp = 37% ± 0,28

ci = 93,33% ± 0,09

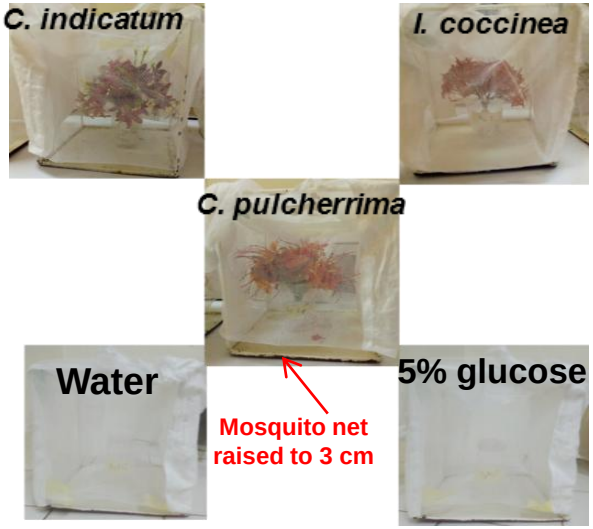
Trophic preference 2/3

Methodology: Feeding behaviour test

Species : *An. coluzzii* and *An. gambiae*

Mosquito dusting

Collection and confection of flowers bouquets



Odour traps

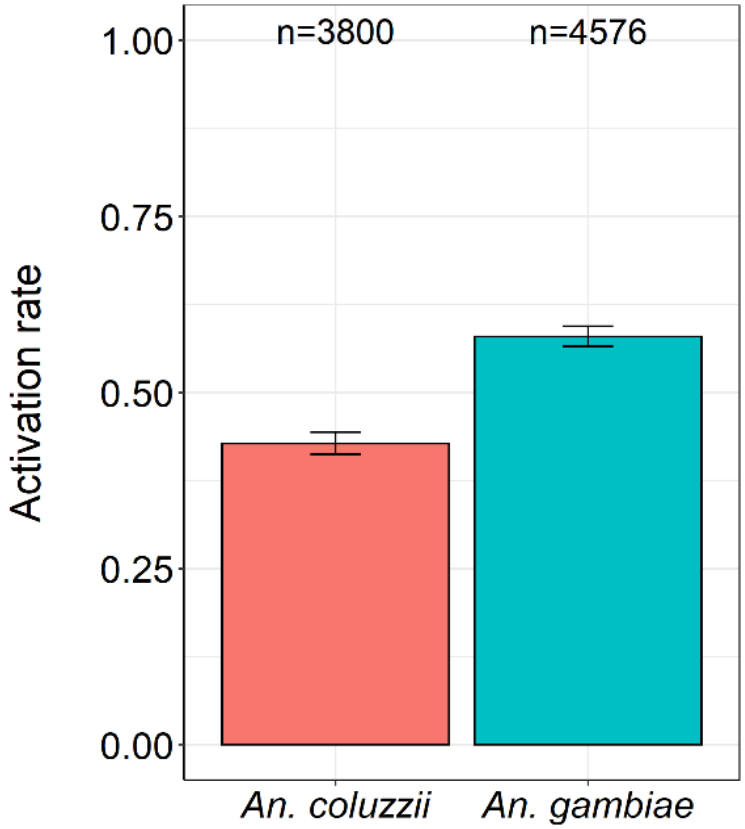
Odour traps in cages

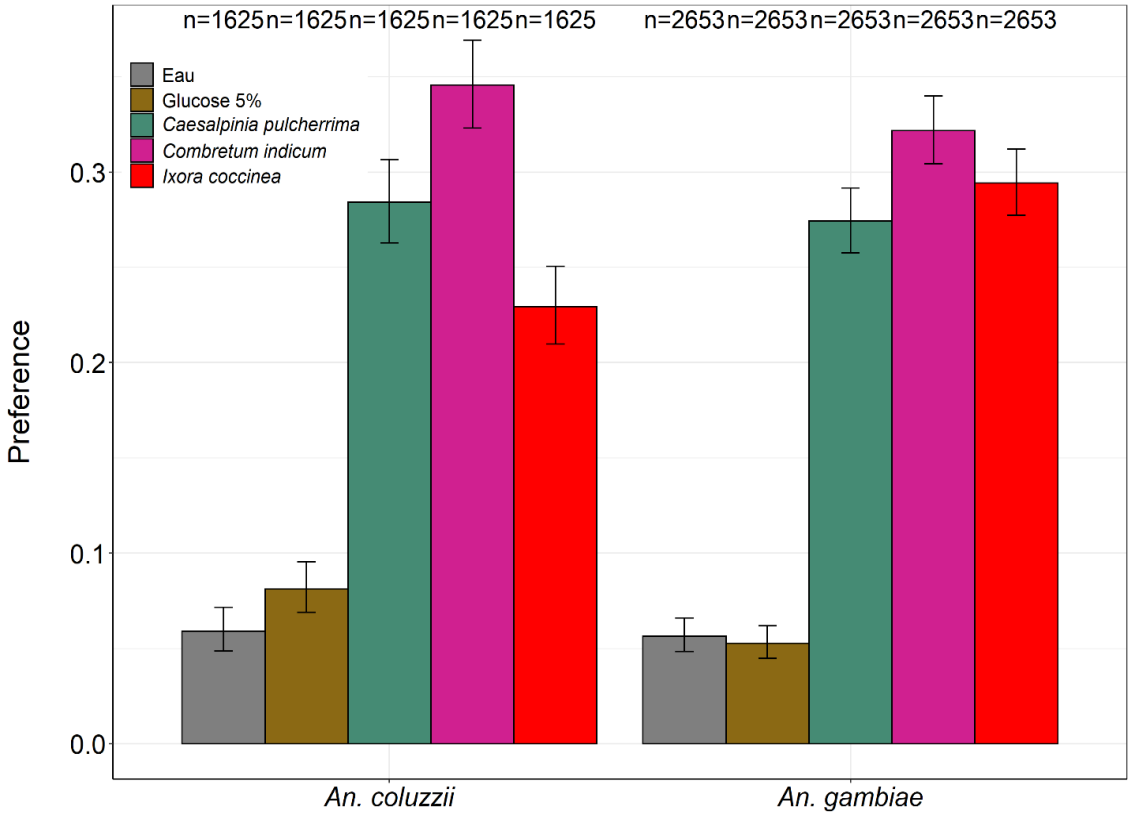
Release of *An. gambiae* and *An. coluzzii* mosquitoes at 6 pm in the experimental cages

Vacuuming at 6 am and counting of mosquitoes per trap

Trophic preference 3/3

Results: Feeding behaviour test





Activation: Number of mosquitoes caught in all traps out of the total number of mosquitoes released

Preference: Number of mosquitoes caught per trap out of the total number of mosquitoes caught in all traps

Species effect: LRT $X^2_1 = 185.14$, $P < 0.001$

Treatment effect : LRT $X^2_4 = 1453.86$, $P < 0.001$

Activation of *An. gambiae* (58% ± 0.02) > *An. coluzzii* (42% ± 0.02)

Looking at different flowers, mosquitoes had a same preference but especially for ***C. indicum***

Bioassay: Effect of sugar plants on mosquito sensibility 1/5

Methodology: choice of plants

Barleria lupulina

Cascabela thevetia

Barleria lupulina* + *Cascabela thevetia

5% glucose

Choice based on the study of Hien et al. (2016) which showed that mosquitoes fed on the sugars of these two plants were involved in malaria transmission

PLOS | PATHOGENS

RESEARCH ARTICLE

Plant-Mediated Effects on Mosquito Capacity to Transmit Human Malaria

Domonbabele F. d. S. Hien[1]*, Kounbobr R. Dabiré[1], Benjamin Roche[2], Abdoulaye Diabaté[1], Rakiswende S. Yerbanga[1], Anna Cohuet[3], Bienvenue K. Yameogo[1], Louis-Clément Gouagna[3], Richard J. Hopkins[4], Georges A. Ouedraogo[5], Frédéric Simard[3], Jean-Bosco Ouedraogo[1], Rickard Ignell[6], Thierry Lefevre[1,3]*

Photo credit: Thierry LEFEVRE

Insecticide : 0.05% deltamethrine

Bioassay: Effect of sugar plants on mosquito sensibility 2/5

Methodology: Feeding mosquitoes and WHO test tube insecticide

Collection of *An. gambiae* larvae

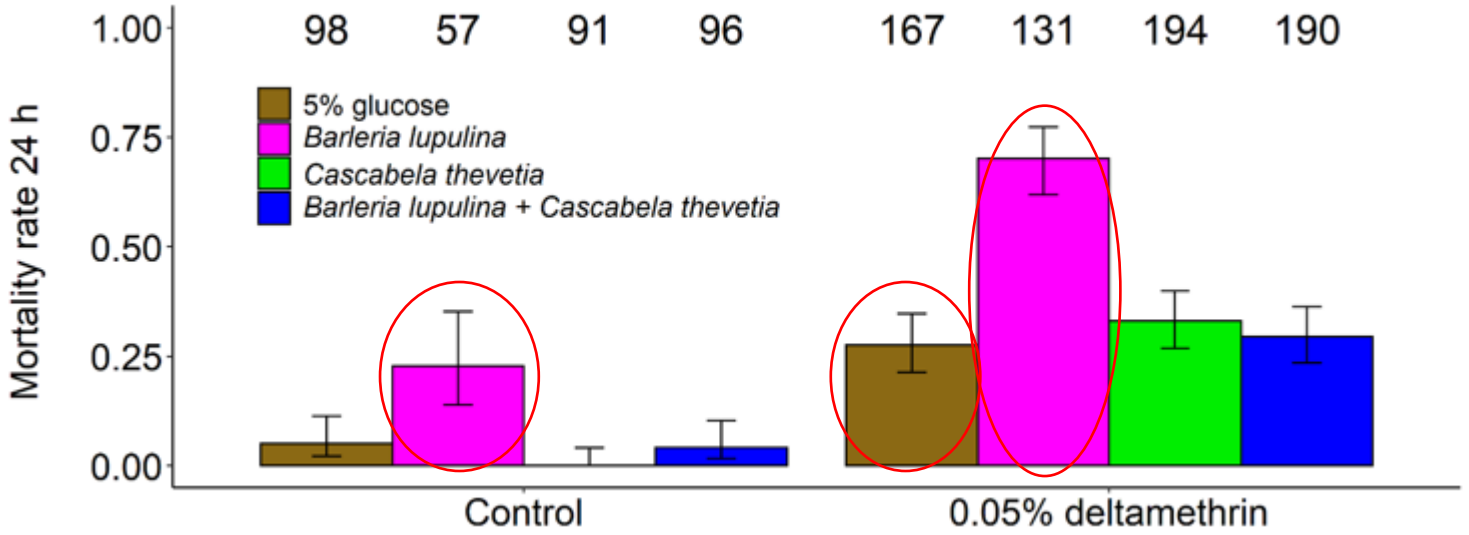
Mosquitoes emerged from larvae collected in the field exposed to sugar diets during 3-5 days

WHO test tube, exposition time 1 h

24-hour mortality reading

Bioassay: Effect of sugar plants on mosquito sensibility 3/5

Results



Treatment effect: LRT X^2_1 = 43.51, P < 0.001

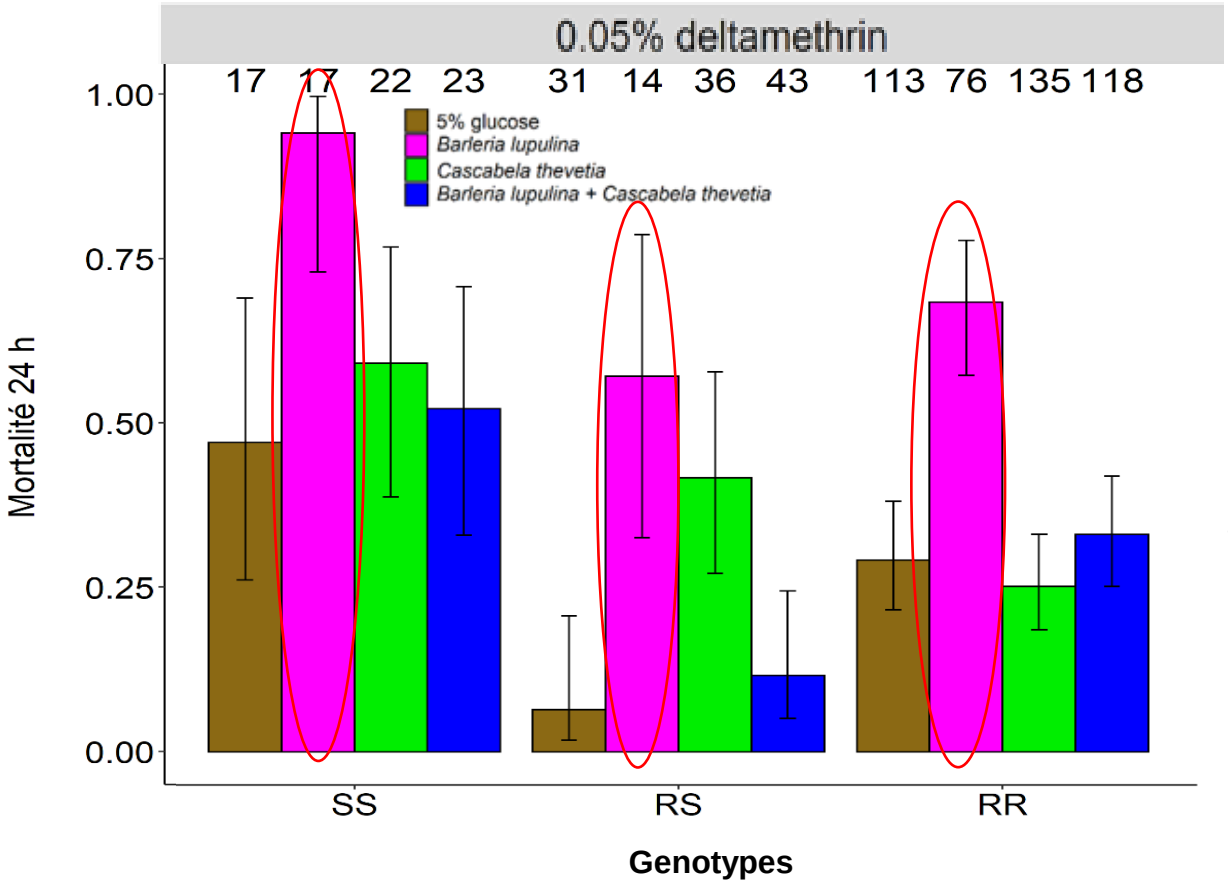
Barleria lupulina diet induced a 2.5 fold-increase in mosquito mortality (70%±0.08) compared to the 5% glucose treatment (28%±0.07)

Contrôle *:* Increased mortality => ***B. lupilina***

No mortality => ***C. thevetia***

Bioassay: Effect of sugar plants on mosquito sensibility 4/5

Results



- Sugar diet*genotypes *kdr* : **LRT X^2_6 = 17.38, P = 0.01**
- => The effect of the sugar diet varies according to the genotype
- For example: RS mosquitoes fed with *B. lupulina* => low mortality compared to SS and RR mosquitoes fed with *B. lupulina*

Figure : Interaction between sugar diet and genotypes *kdr* on the mortality 24 h post-exposition

Bioassay: Effect of sugar plants on mosquito sensibility 5/5

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Natural plant diet impacts phenotypic expression of pyrethroid resistance in *Anopheles* mosquitoes

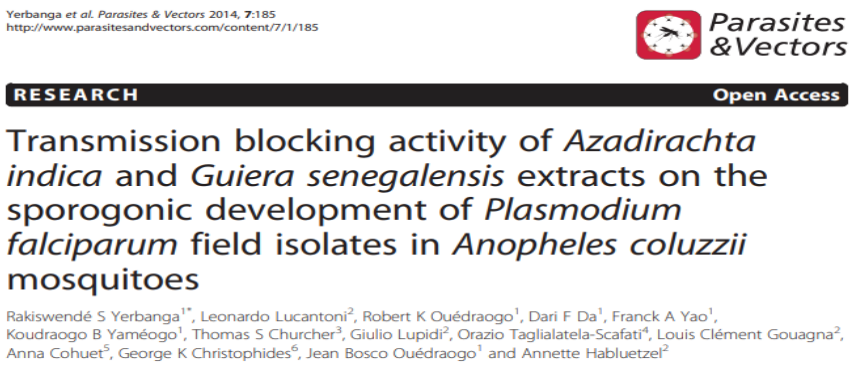
Prisca S. L. Paré[1,2,3✉], Domonbabele F. D. S. Hien[1,2,4], Koama Bayili[1], Rakiswendé S. Yerbanga[1,4,5], Anna Cohuet[2,4], David Carrasco[2], Edwige Guissou[1,2,4], Louis-Clément Gouagna[2], Koudraogo B. Yaméogo[1], Abdoulaye Diabaté[1,4], Rickard Ignell[6], Roch K. Dabiré[1,4], Thierry Lefèvre[2,4,7] & Olivier Gnankiné[3,7]

Extract and molecule 1/8

 Some plant extracts and synthetic molecules can significantly reduce infection

 or block the development of *plasmodium* in malaria mosquitoes.

Demonstration of the anti-plasmodial activity of the commercial extract of NeemAzal(R) in An. coluzzii (Yerbanga *et al*.,2014)



Yerbanga *et al. Parasites & Vectors* 2014, **7**:185
http://www.parasitesandvectors.com/content/7/1/185

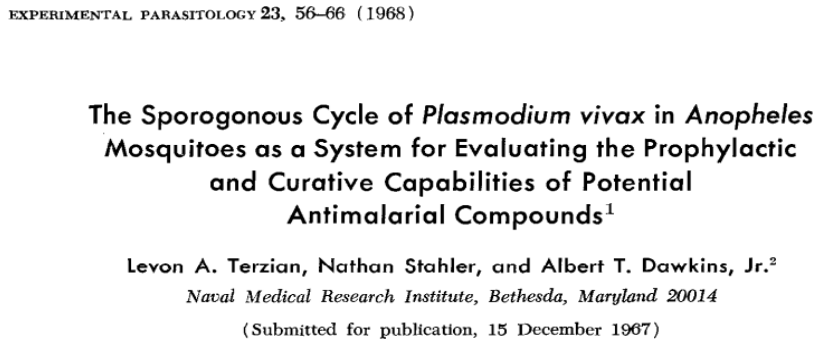
Parasites &Vectors

RESEARCH Open Access

Transmission blocking activity of *Azadirachta indica* and *Guiera senegalensis* extracts on the sporogonic development of *Plasmodium falciparum* field isolates in *Anopheles coluzzii* mosquitoes

Rakiswendé S Yerbanga[1*], Leonardo Lucantoni[2], Robert K Ouédraogo[1], Dari F Da[1], Franck A Yao[1], Koudraogo B Yaméogo[1], Thomas S Churcher[3], Giulio Lupidi[2], Orazio Taglialatela-Scafati[4], Louis Clément Gouagna[2], Anna Cohuet[5], George K Christophides[6], Jean Bosco Ouédraogo[1] and Annette Habluetzel[2]

Proguanil, **pyrimethamine**, **cycloguanil** etc. **=>** block the development of the parasite in the mosquito vectors (Terzian *et al*., 1968)



EXPERIMENTAL PARASITOLOGY 23, 56–66 (1968)

The Sporogonous Cycle of *Plasmodium vivax* in *Anopheles* Mosquitoes as a System for Evaluating the Prophylactic and Curative Capabilities of Potential Antimalarial Compounds[1]

Levon A. Terzian, Nathan Stahler, and Albert T. Dawkins, Jr.[2]
Naval Medical Research Institute, Bethesda, Maryland 20014

(Submitted for publication, 15 December 1967)

Extract and molecule 2/8

Extract

Hien et al. Parasites Vectors (2021) 14:479
https://doi.org/10.1186/s13071-021-04992-z

Parasites & Vectors

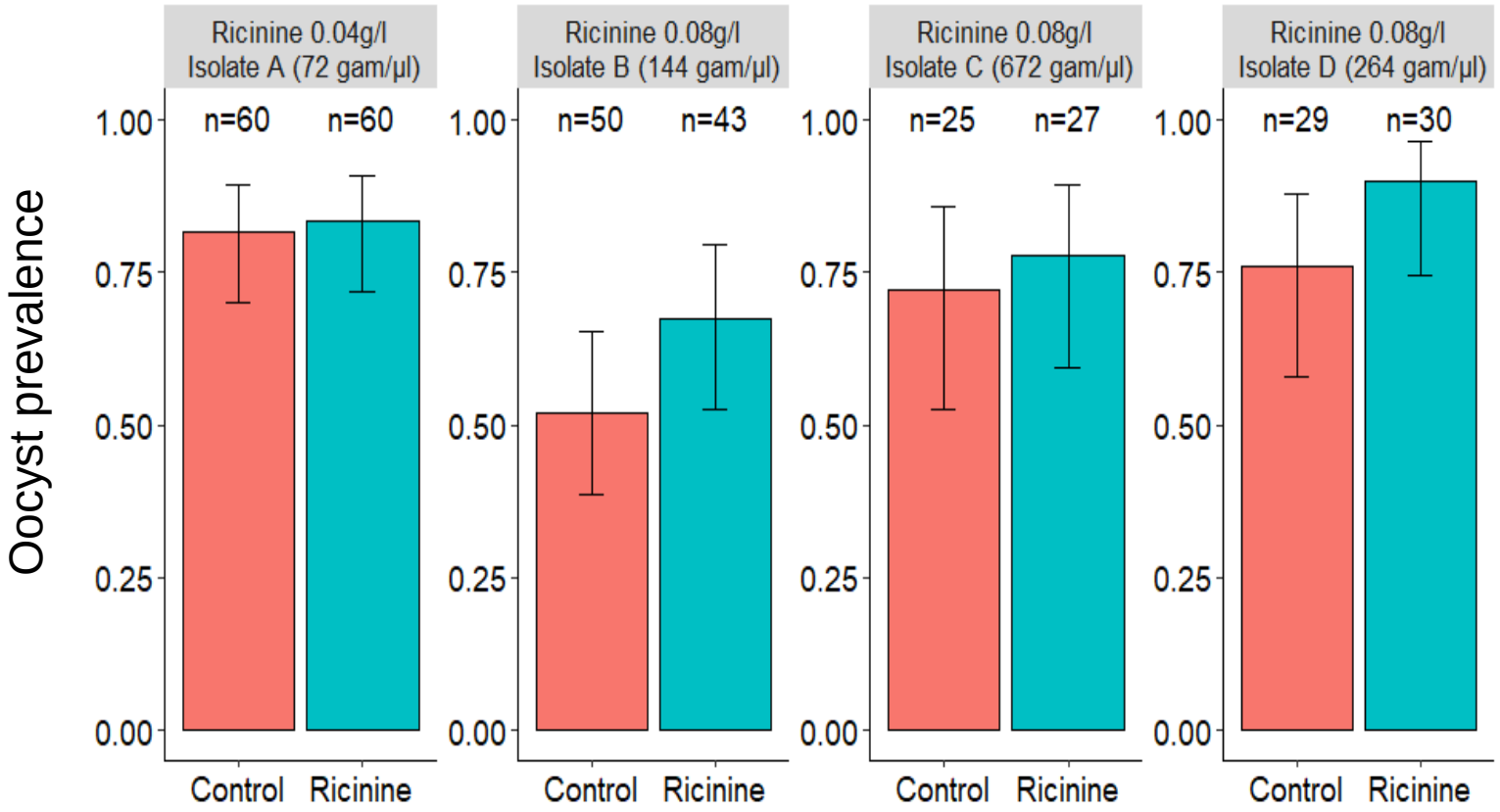
RESEARCH Open Access

Contrasting effects of the alkaloid ricinine on the capacity of *Anopheles gambiae* and *Anopheles coluzzii* to transmit *Plasmodium falciparum*

Domonbabele F. D. S. Hien[1,2,3*], Prisca S. L. Paré[1,3,4], Amanda Cooper[5], Benjamin K. Koama[1,6], Edwige Guissou[1,2,3], Koudraogo B. Yaméogo[1,2], Rakiswendé S. Yerbanga[1,2], Iain W. Farrell[5], Jean B. Ouédraogo[1], Olivier Gnankiné[4], Rickard Ignell[7], Anna Cohuet[2,3], Roch K. Dabiré[1,2], Philip C. Stevenson[5,8] and Thierry Lefèvre[2,3,9]

Extract and molecule 3/8

Extract: results

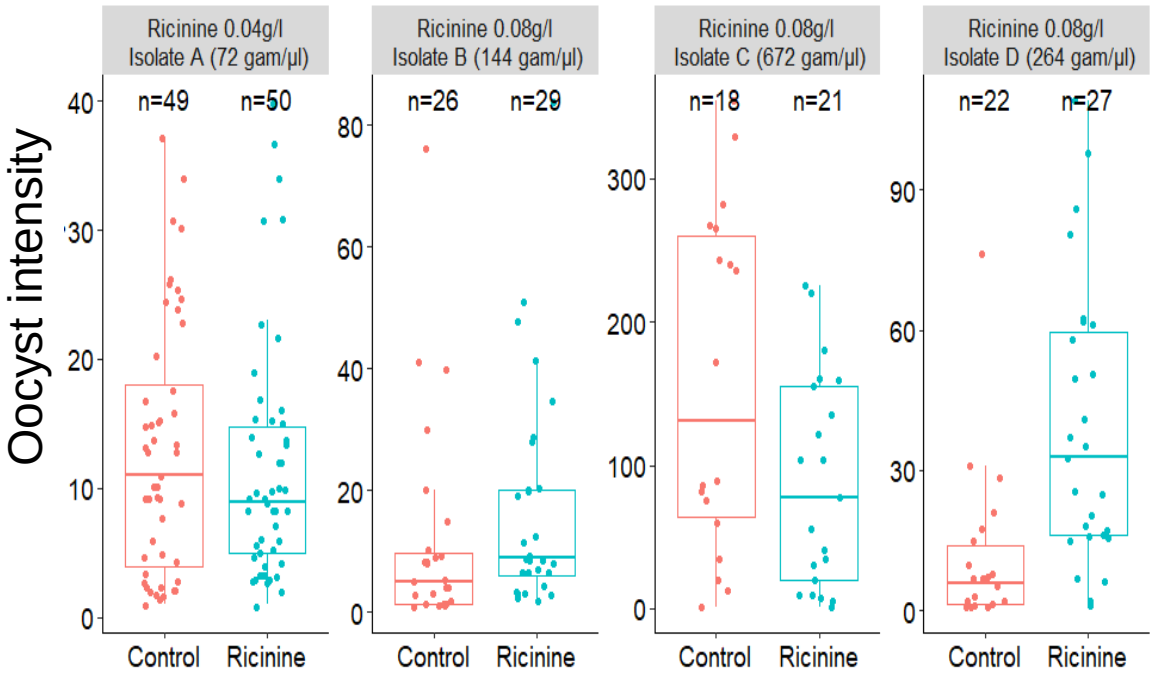


- 0.04 g/l no effect, **(LRT X^2_1 = 0.06, P = 0.81), isolate A**

- However, 0.08g/l ricinine infection rate of 14% (LRT X^2_1 = 4.5, P = 0.03), **isolates B, C and D**

Extract and molecule 4/ 8

Extract: results



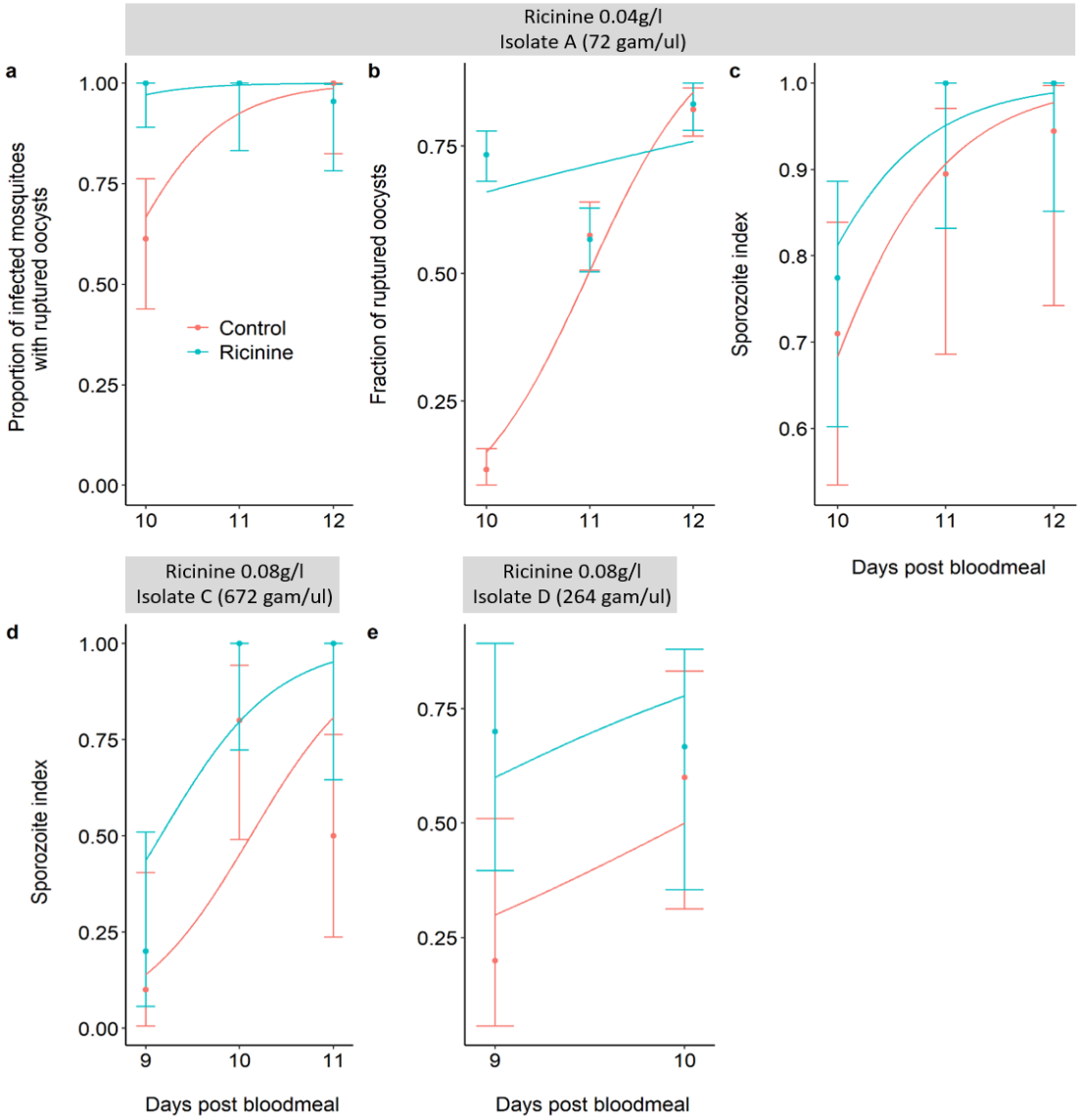
➢0,04 g/l no effect, **(LRT X^2_1 = 0.5, P = 0.48), isolate A**

➢0,08g/l ↗ **(LRT X^2_2 = 16.51, P = 0.0003**

➢Particularly, 0.08g/l increased the number of parasites for isolates B and D in contrast to isolate C

Extract and molecule 5/ 8

Extract: results



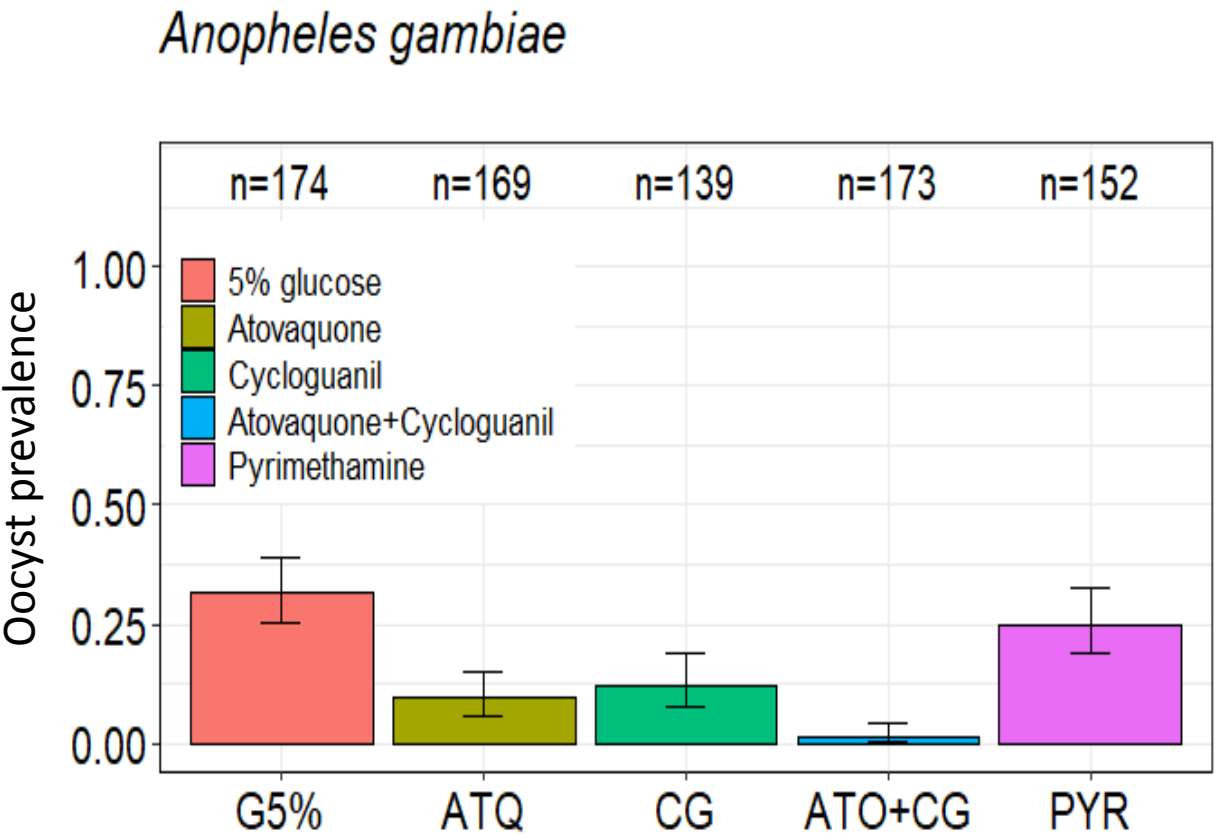
➢Ricinine increases the growth rate of *P. falciparum* **(LRT X^2_1 = 12.8, P = 0.0003, *LRT* X^2_1 = 109, P <0.0001, Figure 2a,b respectively).**

➢sporozoite dissemination in the head/thorax of ricinine-fed mosquitoes occurred earlier than in control (**LRT X^2_1 = 4.36, P=0.037, Figure 2c, d)**.

➢These results suggest that ricinine increased the maturation of *P. falciparum*

Extract and molecules 6/8

Molecules



We found a significant effect of treatment on oocyst prevalence (LRT X^2_4= 31.83 , P < 0.001)

In Particular, ATQ+ CG treatment reduced the number of oocyst

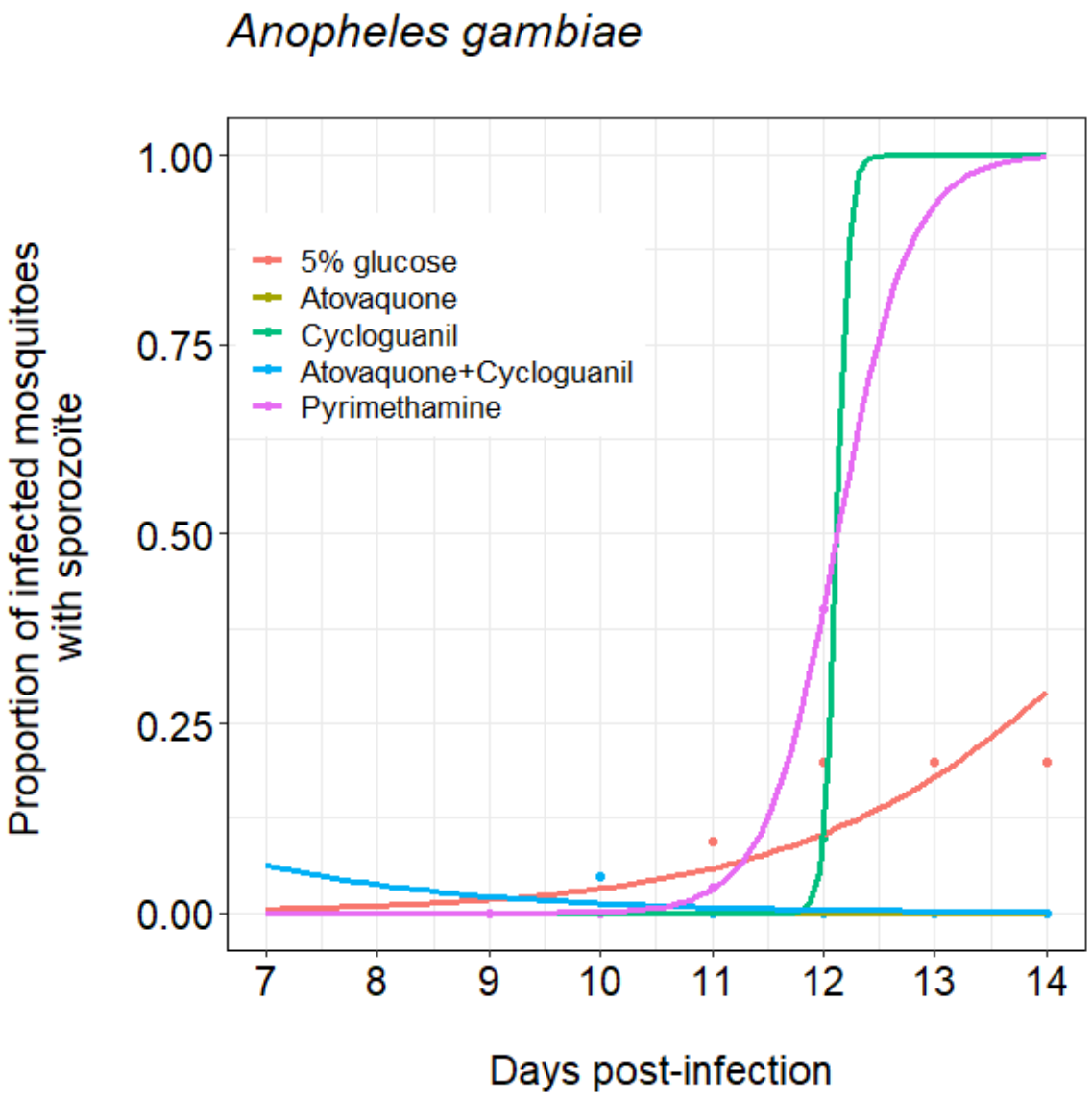
G 5% = 26% > ATO+CG = 1%

CG = 19% > ATO+CG = 1%

PYR = 31% > ATO+CG = 1%

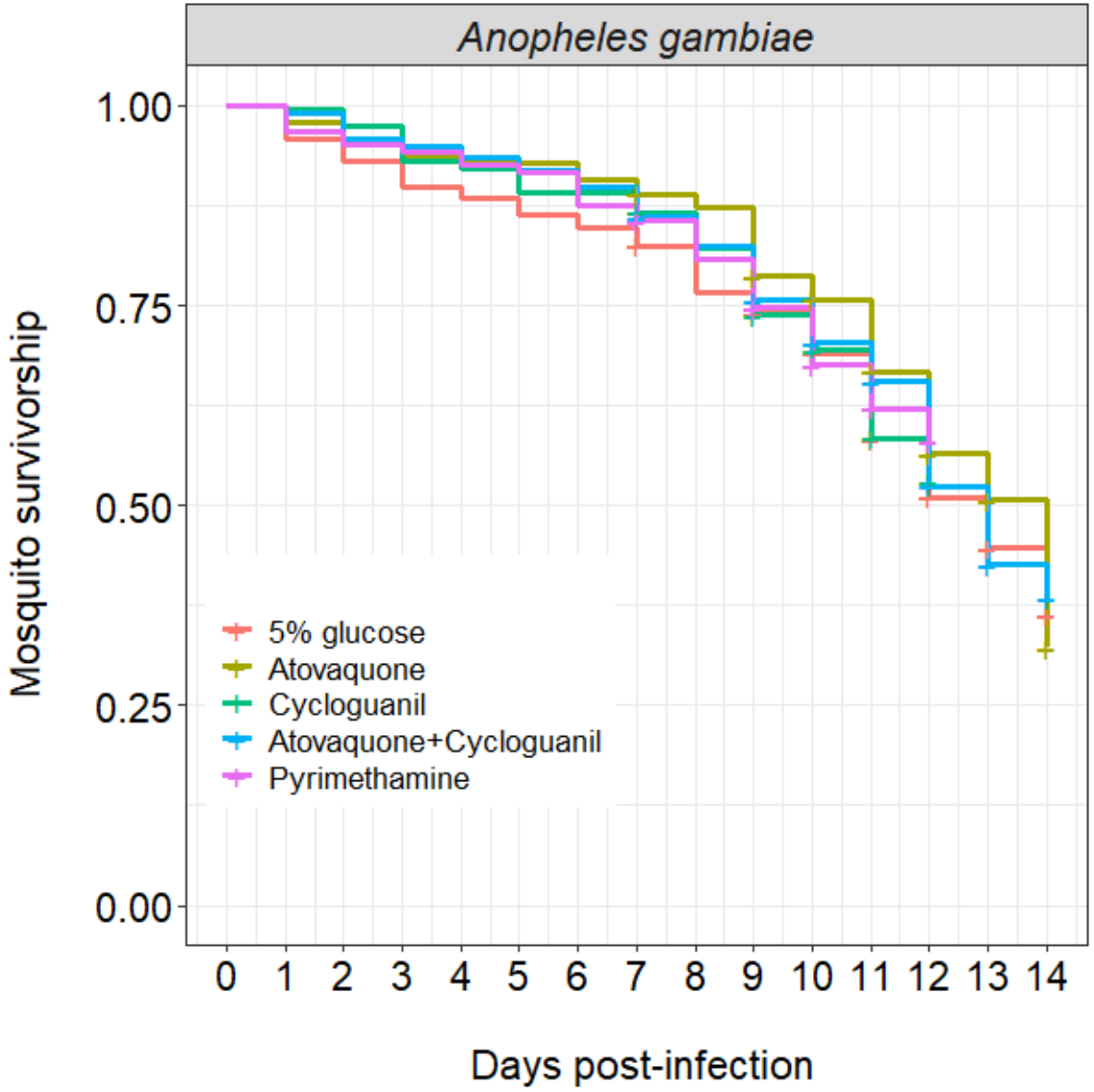
Extract and molecules 7/8

Molecules



We found a significant effect of treatment on sporozoite invasion of mosquito salivary glands
(LRT X^2_4= 19.63 , P < 0.001)

Extract and molecules 8/8



we did not find a significant effect of treatment on mosquitoes survival

LRT X^2_4= 3.05 , P = 0.55

THANK YOU !!!

Role of natural nectar sources on malaria transmission and contribution of vectors to ecosystem services (PALUNEC)

Institut de Recherche pour le Développement

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2019-2022