Infection of the malaria vector Anopheles coluzzii with the entomopathogenic bacteria Chromobacterium sp. Burkina (C.sp_B) highly reduces larval survival and adult reproductive potentials

Edounou Jacques Gnambani
Nazi Boni University
Etienne Bilgo (bilgo02@yahoo.fr)
Institut de Recherche en Sciences de la Santé
Roch K. Dabiré
Institut de Recherche en Sciences de la Santé
Adrien Marie Gaston
Nazi Boni University
Abdoulaye Diabaté
Institut de Recherche en Sciences de la Santé

Research Article

Keywords: Chromobacterium C.sp_B., Survival, Larvae, Insemination Rate, Wings, Anopheles coluzzii, Malaria.

Posted Date: April 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1559168/v1

Abstract

Background

Additional vector control tools are urgently needed to control malaria transmission in Africa. A native strain of *Chromobacterium sp*. from Burkina Faso (*C.sp_B*) was recently characterized. In bioassays, this bacterium showed a promising virulence and against adult mosquitoes and reduces their blood feeding propensity and fecundity. The current study aimed to assess entomopathogenic effects of *C.sp_B* on larval stages of mosquitoes, its impacts on infected mosquitoes reproductive capacity and their offsprings.

Methods

Three different types of bioassays were performed in laboratory. The first bioassay aimed to evaluate the impact of *C.sp_B* infection on mosquito larvae survival using different bacterial concentrations. The second bioassay wanted to access the effect of *C.sp_B* infection on female insemination rates. The third bioassay investigated the impact of a non-lethal exposure of C.sp_B to parent mosquitoes on the body size of its offsprings. During bioassays mosquitoes were infected through the well-established systems of larvicidal procedure and through cotton ball soaked with 6% glucose containing.

Results

Burkina Faso native strain of *Chromobacterium sp (C.sp_B).* killed larvae of the pyrethroid resistant *Anopheles coluzzii* with LT80 of ~0.60 ± 0.03 days at 10^8 CFU/ml at larval breeding trays. Interestingly, this bacterium had important effects on mosquito reproductive success by reducing the insemination rate of uninfected females from 95 ±1.99 % to 21 ±3.76 % for the infected females. Moreover, *C.sp_B* considerably reduced the wing size of offspring from infected parents compared to uninfected parents. Within females, the wing size was 2104.157 ± 20.89 mm and 2547.569 ± 17.08 mm for offspring from *C.sp_B.* infected females and uninfected females respectively. For males the size of wings were respectively, 1991.059 ± 14.98 mm and 2436.220 ± 13.52 mm for offspring from *C.sp_B* infected males and uninfected males respectively

Conclusion

This study showed that the Burkina Faso *Chromobacterium sp* strain *(C.sp_B)* was highly virulent against larvae of insecticide-resistant *An. coluzzii*, and reduced both mosquito reproduction capacity and offspring fitness. However, additional lab, field, safety and social acceptance studies are needed to withdraw more conclusions about the practical utility of this promising bacterial strain for malaria vector control.

Background

Chemical insecticide application is still the most common method for mosquito vector control [1]. Recent evidence suggests that progress in global malaria control has stalled, with an estimated 229 million malaria cases in 2019 in 87 malaria endemic countries, and an increase in malaria incidence in the region of Africa [2]. This stall in progress overlaps with increasing reports of insecticide resistance, which poses a growing challenge to malaria vector control programs. For insecticide resistance management, scientists should develop and implement a comprehensive plan for global, regional and national plan to address insecticide resistance [1, 2]. It becomes crucial to develop new vector control tools soon that will provide further options for insecticide resistance management In the past decade, there has been renewed interest in the use of the biological vector control strategies which aim to suppress or decrease insect pest populations by introducing symbiotic bacteria into wild populations [3–6]. A lot of approaches are focusing on the development of natural microorganisms or genetically engineered microorganisms to either block the development of the malaria parasite within the Anopheles vector [7–9], or to kill the vector itself [8, 10, 11]. Despite intensive efforts to develop entomopathogenic microorganisms as biocontrol agents against malaria vectors, most of the strains under investigation have not met expectations due to some functional and practical limitations [12]. For example, bacteria such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) show no residual persistence post-application [13]. Interestingly new promising microbial-based control tools such as the use of the bacteria Wolbachia and some Microsporidia (MB) are under investigation for malaria control [14–16]. However these results are from laboratory and several development steps need to be complete before these tools can reach public heath area for malaria control. Among promising microbial-based malaria vector control tools, some members of the genus *Chromobacterium sp.* such as *Chromobacterium vaccinii* and *Chromobacterium sp. Panama* (*Csp_P*) have shown to have insecticidal activity across different species of mosquitoes including *Aedes Aegypti* and *Anopheles gambiae s.s.* [8]. Additionally, *Caragata et al.*, have recently demonstrated that a non-live preparation of *Csp_P* was a highly effective larval mosquito biopesticide [8]. Despite intensive efforts to develop entomopathogenic *Chromobacterium* as biocontrol agents against malaria vectors, the vast majority of strains under investigation are isolated outside of malaria endemic regions as Africa to our knowledge. We hypothesized that development of *Chromobacterium* based strategy for biological control of malaria will need to preferentially employ local isolates, as these will be adapted to kill local mosquitoes and survive local conditions (i.e. rainy season heat, sunlight and humidity) better than imported strains. In this perspective, a new strain of *Chromobacterium sp.* from Burkina Faso formerly identified as *Chromobacterium violaceum* was isolated in Burkina Faso [10]. In the laboratory, the infection of field insecticide-resistant malaria vector *Anopheles coluzzii* with this new strain of *Chromobacterium* has shown high mortality. This Burkinabe new strain reduced mosquito blood feeding propensity and fecundity [10]. The preliminary whole genome sequencing data and the establishment of phylogenic trees have clearly shown that the Burkinabe strain of *Chromobacterium* is not matching with any declared specie of *Chromobacterium sp.* For the purpose of the current study and waiting completing of supplementary analysis for new specie declaration the name *Chromobacterium sp. Burkina* (*C.sp_B*) will be used to call this strain. In the present study, we further explored the mosquitocidal properties of *C.sp_B* against larval stages of malaria vectors Anopheles coluzzii. We also

investigated the impact of *C.sp_B* on reproductive traits within adult mosquitoes and its transgenerational impacts on fitness parameters of mosquitoes.

Materials And Methods

Mosquito colonies, maintenance and PCR determination of kdr levels

For bioassays, F1 progeny of *An. coluzzii* reared from larval collections at Kou Valley (11°23' N, 4°24' W). Mosquitoes from these areas are highly resistant to multiple insecticides currently used for malaria control [18]. Emerged adult mosquitoes from pupae of this first generation were immediately sexed in order to avoid any mating. Then, virgin males and females were kept in separate 30×30×30 cm cages. Sterile cotton, filter paper, and sterilized nets were used to maintain the cages as sterilely as possible. Only non-blood-fed females, 2–5 days old, were used for bioassays. All bioassays were carried out at 25 ± 2 °C and 80 ± 10% relative humidity. The *kdr* gene prevalence within a subsample of mosquitoes (210 mosquitoes) was performed using the PCR protocol and primer sequences previously described [19]. The mutation L1014F was only analysed because it is the commonest in West Africa, whereas the L1014S mutation is confined to East Africa [19].

Bacterial strain

Gnambani *et al.*, described previously the same strain as a *Chromobacterium violaceum* specie based on the VITEK2 system identification. However the GBDP phylogenetic tree based on whole genome sequences of Burkina Faso *Chromobacterium sp*. strain (*C.sp_B*) has shown that the isolate is indeed a new specie, closely related to *C. haemolyticum*. *C.sp_B* and *C. haemolyticum* have 66.4% homology using the dDDH algorithm d4. The tree also told us that it is not closely related to *C. violaceum*. Importantly, the tree has shown that *C.sp_B* and *Chromobacterium sp. Panama* (*Csp_P*) another promising mosquitocidal bacterium (Ramirez *et al.*, 2014) is different even if *Csp_P* is not officially declared specie. In addition to this ongoing genomic data, future phenotypic characterizations on this *C.sp_B* will be performed for the official new strain declaration and registration.

Bacterial infection formulation

Mosquitoes used for bioassays were not treated with antibiotics. They were maintained on 6% glucose for 2–5 days post emergence. Mosquitoes were then starved overnight and fed for 24 hours on cotton balls moistened with a 6% glucose solution containing *C.sp_B* at desired concentrations (bacterial cells (CFU) / ml) regarding the purposes of the bioassays. The detailed protocol of bacterial formulation and exposure of mosquitoes is described by Gnambani et al. [10].

Exposure of *Anopheles coluzzii* larvae to *C.sp_B*

For this bioassay, an overall of 600 L3 *An. coluzzii* larvae collected from breeding sites at Valley du Kou (11°23' N, 4°24' W) were exposure to *C.sp_B* at five serial dilution concentrations from 10^8 to10^4

CFU/ml. The levels of *kdr* mutations within a subsample of these larvae were also performed. During these bioassays, larvae were bred in with TetraMin® *ad libitum* in individual cup per treatment and per concentration. Dead larvae were recorded daily over 3 days before pupal stage.

Effect of *C.sp_B* on the reproductive fitness of adult mosquitoes

To assess the impact of *C.sp_B* on mosquito reproductive fitness two different bioassays were carried out: Firstly, the effect of *C.sp_B* exposure on insemination rate female mosquitoes and secondly the impact of *C.sp_B* exposure on mosquito offspring body size by measuring their wing lengths.

Effect of *C.sp_B* on insemination rate female mosquitoes

3-5 days virgin males and females of *An. coluzzii* were exposed to feed upon cotton balls moistened with a 5% glucose solution containing 10^6 CFU / ml in a cage (30×30×30cm) for 24 hours. The control group mosquitoes were not exposed to sterile 5% glucose solution for 24 hours without any bacteria. In order to access the impact of *C.sp_B* on the insemination rate, the following crossing were performed (Table 1)

Table 1: Crossing between males and females based on *C.sp_B* infection status

	Infected Females (IF),	non-Infected Females (nIF)
Infected Males (IM)	IF X IM	nIF X IM
non-Infected Males (nIM).	IF X nIM	nIF X nIM

Legend: IM= Infected Males; IF= Infected Females; nIM= non-Infected Males and nIF= non-Infected Females

Overall 360 mosquitoes per crossing type with a sex ratio of 2:1 (240 males for 120 females) were allowed to mate at three mating times: For 30 minutes, 60 minutes and 24 hours in 30×30×30 cm cages. Following the three mating times, 120 females were withdrawn in each cage. Twenty-four hours later, spermathecae of female mosquitoes per crossing type were dissected and the insemination status was assessed under microscope at the magnification of X400.

Effect of *C.sp_B* infection on body size of offsprings from infected mother mosquitoes

This third assay aimed to assess the effect of *C.sp_B* infection on body size of offsprings from infected female mosquitoes through the measurement of the lengths of wings. Three hundred selected L3 larvae from eggs oviposited by uninfected female versus *C.sp_B* infected were placed in larval bowls containing 800 ml of distilled water. Notice that for infections inseminated female mosquitoes were exposure to *C.sp_B* at 10^6 CFU/ml. Larvae were also reared at standard condition in order to avoid differences due to biotic and abiotic fluctuations. Wing lengths (left and right) were measured as described previously [20] Both wings were dissected, mounted dried on microscope slides, and photographed with Leica EZ4 D

(Leica Microsystems, Suisse). Their size was then measured using the software Image J1.41.0 (Wayne Rasband, National Institutes of Health, U.S.A.) from the annular notch to the end of the radius vein (excluding fringe scales). This length raised to the cube (WL3) was considered an index of mosquito size.

Data analysis

Data were entered into Microsoft Windows Excel 2013, checked for accuracy, and then imported to R studio version 3.2.0 for data manipulation, visualization and statistical analysis (Additional file 2. Fisher's exact test, $P < 0.05$ was accepted for statistically significant. LT_{80} survival for treatments and concentrations were determined using generalized linear model (GLM) approach. For all bioassays, mosquitoes were considered alive if they could stand upright and dead if they were unresponsive to stimuli following the 2013 recommendations by the WHO Pesticides Evaluation Scheme [21].

Results

Entomopathogenic effect of C.sp_B on Anopheles coluzzii larvae survival

Within ~ 0.60 ± 0.03 days post-infection, more than 80% of mosquitoes exposed to the higher concentration to 10^8 bacterial cells / ml were dead, so significantly faster ($P < 0.05$) than those exposed to the 3 lower concentrations (Fig. 1, Table 2). We also observed a difference ($P < 0.05$) in term of virulence (LT_{80}) between 10^7 and 10^6 bacterial cells / ml with LT_{80} values of 0.80 ± 0.07 and 1.14 ± 0.06 days respectively (Table 2). The lower concentration 10^4 bacterial cells / ml did not reach the LT_{80} threshold out of ~ 2.38 ± 0.18 days. Observing the survival over 3 days, larvae of uninfected control group never dropped below 93.5% of survival rate (Fig. 1).

Table 2
LT_{50} and LT_{80} survival values of *Anopheles coluzzii* laboratory L3 larvae treated by *C.sp_B* reared under standard insectary conditions during 3.5 days.

Treatments	LT_{50} Mean	SE	LT_{80} Mean	SE
Control	-	-	-	-
P4	-	-	2.38	0.18
P5	2.80	0.03	1.55	0.03
P6	2.25	0.07	1.14	0.06
P7	1.67	0.08	0.80	0.07
P8	1.12	0.04	0.60	0.03

Legend: Control = Control is exposed to any treatment; P4 = 10^4 bacteria cells / ml, P5 = 10^5 bacteria cells/ml, P6 = 10^6 bacteria cells / ml; P7 = 10^7 bacteria cells/ml and P8 = 10^8 bacteria cells / ml; SE = standard error of the mean; Pairwise comparison of LT_{50} and LT_{80} values per conidia suspension concentrations: All treatments were significant at $p < 0.05$.

Effect of C.sp_B on insemination rate female mosquitoes

The highest insemination rate was recorded in females from the crossings involving uninfected males and females (Fig. 2), which is showing at insemination between (95 ± 1.99% and 75 ± % 3,95). The lowest insemination rates were observed from the crossings between *C.sp_B* infected males and females with insemination rates between 35 ± 4.35% and 21 ± 3.76%. Notice that the crossing time did not show any significant differences of insemination rates within different treatments (ANOVA, df = 6, P = 0.2436). Regardless of the different treatments, significantly differences in insemination rates were observed (ANOVA, df = 3, P < 0.001**).** The average of interactions showed statistically significant differences for most of the treatments (Table 3).

Table 3
Interaction average of mosquito insemination rate between different crossings based on *C.sp_B* infection

Interaction_average	P-value
IM_nIF - IM_IF	0.00876**
nIM_IF - IM_IF	< 0.001***
nIM_nIF - IM_IF	< 0.001***
nIM_IF - IM_nIF	0.60927
nIM_nIF - IM_nIF	< 0.001***
nIM_nIF - nIM_IF	< 0.001***
**: Statistically significant	
Legend: IM = Infected Males; IF = Infected Females; nIM = non-Infected Males and nIF = non-Infected Females.	

The female insemination rate was assessed during three different contact times (30 minutes, 60 minutes and 24 hours).

Effect of C.sp_B infection on wing length of offsprings from infected mother mosquitoes

Overall for both females and males, the wing sizes of the uninfected mosquito size were significantly bigger than *C.sp_B* infected mosquito (Fig. 4, chi-squared = 166.01, df = 3, P < 0.001). Within females, the average of wing size was 2104.157 ± 20.89 mm and 2547.569 ± 17.08 mm for offspring from *C.sp_B* infected females and uninfected females respectively. For males the average of the wing size was 1991.059 ± 14.98 mm and 2436.220 ± 13.52 mm for offspring from *C.sp_B* infected males and uninfected males respectively (Fig. 3).

The exposure of L3-larvae insecticides resistant *Anopheles coluzzii* to different concentrations of Burkina Faso local and new strain of *C.sp_B* has shown high rates of mortality within mosquitoes. This strain has previously shown a high virulence against adults [10]. The larvicidal activity of our local strain of

Chormobacterium could be the direct result of a mosquitocidal factor or systemic infection through dissemination into the hemolymph. Alternatively, *C.sp_B* colonization of the midgut might cause mortality indirectly by interfering with vital functions of the mosquito [8]. Among the potential virulence factors, which may contribute to mosquitocidal effect, the production of the violacein, siderophores, hydrogen cyanide, and chitinases could be cited [9]. In addition, some strains *Chromobacterium* are capable of forming biofilms in vitro, though whether biofilm formation occurs within the mosquito midgut remains untested.

Another entomopathogenic effect that *C.sp_B* has shown was the important reduction of insemination rates of females. The insemination rates generated by crossing of infected males and females was similar to those obtained by Helinski *et al*. in large cages with the classical Sterile Insect Techniques by lower irradiation dose [22]. With *C.sp_B* infected males, the sperm production can also be negatively affected by bacterial treatments. The low insemination rates could be also due to the alteration of energy reserves, and reduction of mating capacity from *C.sp_B* infected mosquitoes. Flight and hearing capabilities, pheromone and sperm production, may be responsible for the drop in mating efficiency and for lower insemination rates for some mosquitoes [23].

The body size reduction is another trait, which is associated with mosquito mating capacity and competitiveness success. Our results showed that entomopathognic bacteria *C.sp_B* uninfected *An. coluzzii* offsprings were significantly bigger than infected ones. It was shown previously that pigments and peptides as violacein, siderophores, hydrogen cyanide, and secreted chitinases could affect the fitness of adult mosquitoes [8, 10]. To a certain extent as ss other microorganisms such as Wolbachia, which manipulate the reproduction system of their host, our local strain of *C.sp_B* could be considered as a parasite of mosquito reproduction. By projecting forward in addition to its larvicidal activity *C.sp_B* could be used as a Sterile Insect Technique tool.

Conclusion

The present study shows that the new strain of Chromobacterium from Burkina Faso *C.sp_B* is highly virulent against larvae of wild caught malaria vector *Anopheles coluzzii*. The study also shows that this bacterium has important pathogenic effect on mosquito mating success and competitiveness mainly a reduction of female insemination rates. Finally this bacterium shows trans-generation impacts through a reduction of offspring mosquito sizes from infected parents. From our data, this local strain of Chromobacterium *C.sp_B* from Burkina Faso is a promising tools for malaria vector control at both larval and adults stages. However additional studies need to be complete to withdraw more conclusions on the practical utility of this bacterium for malaria control. Future extensive studies will focus on development of disseminating methods of *C.sp_B* to wild mosquitoes, its safety to human and the environment but also the social acceptances of this bacteria.

Abbreviations

C. violaceum: *Chromobacterium violaceum*; *C.sp_B: Chromobacterium sp. Burkina* LT_{50}: Is the median (50%) Lethal Time (time until death) after exposure of a mosquito to bacterial infections; LT_{80}: Is the 80% Lethal Time (time until death) after exposure of a mosquito bacterial infections; IM= Infected Males; IF= Infected Females; nIM= non-Infected Males and nIF= non-Infected Females.

Declarations

Acknowledgements

We are very grateful to Saré Issiaka, Rosalie Some, and Lamy Doube Lucien for their technical contributions to the field and lab works.

Experiments with animals were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. In addition, Experiments followed the IRSS Animal Welfare Assurance A5926-01. Trained personnel and veterinarians cared for animals involved in this study and all efforts were made to minimize suffering. All works with *C. violaceum* were performed under biosafety containment level II requirements.

Authors' contributions

EB, AD, AMGB, AS, RKD and EJG designed the experiments; EJG and EB performed the experiments and analyzed the data. EJG and EB and wrote the manuscript. EB and AD are guarantors of the study. All authors read and approved the final manuscript.

Funding

This work was partially supported by the Burkina Faso government PhD thesis Scholarship granted to Edounou Jacques Gnambani. The FNIH-Wellcome Trust International Training grant reference Ref: 218771/Z/19/Z awarded to Dr. Etienne Bilgo also supported purchasing some lab and field reagents during this study

Availability of data and materials

The supplementary R codes and data for all analyses in this article are in supplemental files and could also be available upon request to the corresponding authors.

Consent for publication

All authors have approved the final manuscript and consent for the publication.

Competing interests

The authors declare no competing financial interests.

Author details

[1] Institut de Recherche en Sciences de La Santé (IRSS) Direction Régional de l'Ouest (DRO) / CNRST, [2] Institut National de Santé Publique (INSP) / Centre Muraz, Bobo Dioulasso, Burkina Faso, [3] Centre d'Excellence Africain en Innovation Biotechnologiques pour l'Elimination des maladies à Transmission Vectorielle (CEA-ITECH/MTV)/ University Nazi Boni, [4] Université Nazi Boni (UNB), Bobo-Dioulasso, Burkina Faso

References

1. OMS. Paludisme. (2019) doi:https://www.who.int/malaria/media/world-malaria-report-2019/fr/.
2. World Health Organization. *WHO World Malaria Report 2020*. *Malaria report 2020* (2020).
3. Wang, S. *et al.* engineered symbiotic bacteria. **1402**, 1399–1402 (2017).
4. Wang, S. *et al.* Fighting malaria with engineered symbiotic bacteria from vector mosquitoes. (2012) doi:10.1073/pnas.1204158109.
5. Riehle, M. A., Moreira, C. K., Lampe, D., Lauzon, C. & Jacobs-Lorena, M. Using bacteria to express and display anti-Plasmodium molecules in the mosquito midgut. *Int. J. Parasitol.* **37**, 595–603 (2007).
6. Tchioffo, M. T. *et al.* Midgut Microbiota of the Malaria Mosquito Vector Anopheles gambiae and Interactions with Plasmodium falciparum Infection. **8**, (2012).
7. Gao, H. *et al.* refractoriness to Plasmodium infection via. *Nat. Microbiol.* **6**, (2021).
8. Ramirez, J. L. *et al.* Chromobacterium Csp_P Reduces Malaria and Dengue Infection in Vector Mosquitoes and Has Entomopathogenic and In Vitro Anti-pathogen Activities. *PLoS Pathog.* **10**, (2014).
9. Short, S. M., Van Tol, S., MacLeod, H. J. & Dimopoulos, G. Hydrogen cyanide produced by the soil bacterium Chromobacterium sp. Panama contributes to mortality in Anopheles gambiae mosquito larvae. *Sci. Rep.* **8**, 1–13 (2018).
10. Gnambani, E. J., Bilgo, E., Sanou, A., Dabiré, R. K. & Diabaté, A. Infection of highly insecticide resistant malaria vector Anopheles coluzzii with entomopathogenic bacteria Chromobacterium violaceum reduces its survival , blood feeding propensity and fecundity. *Malar. J.* 1–10 (2020) doi:10.1186/s12936-020-03420-4.
11. Short, S. M., Van Tol, S., Smith, B., Dong, Y. & Dimopoulos, G. The mosquito adulticidal Chromobacterium sp. Panama causes transgenerational impacts on fitness parameters and elicits xenobiotic gene responses. *Parasites and Vectors* **11**, 1–15 (2018).
12. Thomas, M. B. Biological control of human disease vectors : a perspective on challenges and opportunities. *BioControl* (2017) doi:10.1007/s10526-017-9815-y.
13. Tetreau, G. *et al.* Larval midgut modifications associated with Bti resistance in the yellow fever mosquito using proteomic and transcriptomic approaches. *BMC Genomics* **13**, (2012).

14. Herren, J. K. *et al.* A microsporidian impairs Plasmodium falciparum transmission in Anopheles arabiensis mosquitoes. *Nat. Commun.* doi:10.1038/s41467-020-16121-y.
15. Shaw, W. R. *et al.* Wolbachia infections in natural Anopheles populations affect egg laying and negatively. (2016) doi:10.1038/ncomms11772.
16. Baldini, F. *et al.* Evidence of natural Wolbachia infections in field populations of Anopheles gambiae. 1–7 (2014) doi:10.1038/ncomms4985.
17. Ã, L. L. K. *et al.* Violacein synergistically increases 5-fluorouracil cytotoxicity , induces apoptosis and inhibits Akt-mediated signal transduction in human colorectal cancer cells. **27**, 508–516 (2006).
18. Baldet, T., Diabate, A., Namountougou, M., Martin, T. & Dabire, R. K. Multiple Insecticide Resistance in Anopheles gambiae s . l . Populations from Burkina Faso , West Africa. **7**, (2012).
19. Carnevale and Robert, V. *Les anophèles Biologie, transmission du Plasmodium et lutte anti vectorielle. Ed., IRD.* (2009).
20. Hamidou Maïga, Roch K. Dabiré, Tovi Lehmann, FrédéricTripet, and Abdoulaye Diabaté, Variation in energy reserves and role of body size in the mating system of Anopheles gambiae, J Vector Ecol., 2012 Dec;37(2):289-97. doi: 10.1111/j.1948-7134.2012.00230.x. (2012).
21. CS= micro-encapsulated formulation; DT = tablet for direct application; EC= emulsifiable concentrate; EW = emulsion, oil in water; GR = Granule; LN = long-lasting insecticidal net; SC = suspension concentrate; WG = water dispersible granule. * WHOPES - Ph. 2013 (2013).
22. Zhu, K. Y., Heise, S., Zhang, J., Anderson, T. D. & Starkey, S. R. Comparative studies on effects of three chitin synthesis inhibitors on common malaria mosquito (diptera: Culicidae). *J. Med. Entomol.* **44**, 1047–1053 (2007).
23. Helinski, M. E. H. *et al.* Towards a sterile insect technique field release of Anopheles arabiensis mosquitoes in Sudan: Irradiation, transportation, and field cage experimentation. *Malar. J.* **7**, 1–10 (2008).
24. Poda, S. B. *et al.* Impact of irradiation on the reproductive traits of field and laboratory An. arabiensis mosquitoes. *Parasites and Vectors* **11**, 1–12 (2018).

Figures

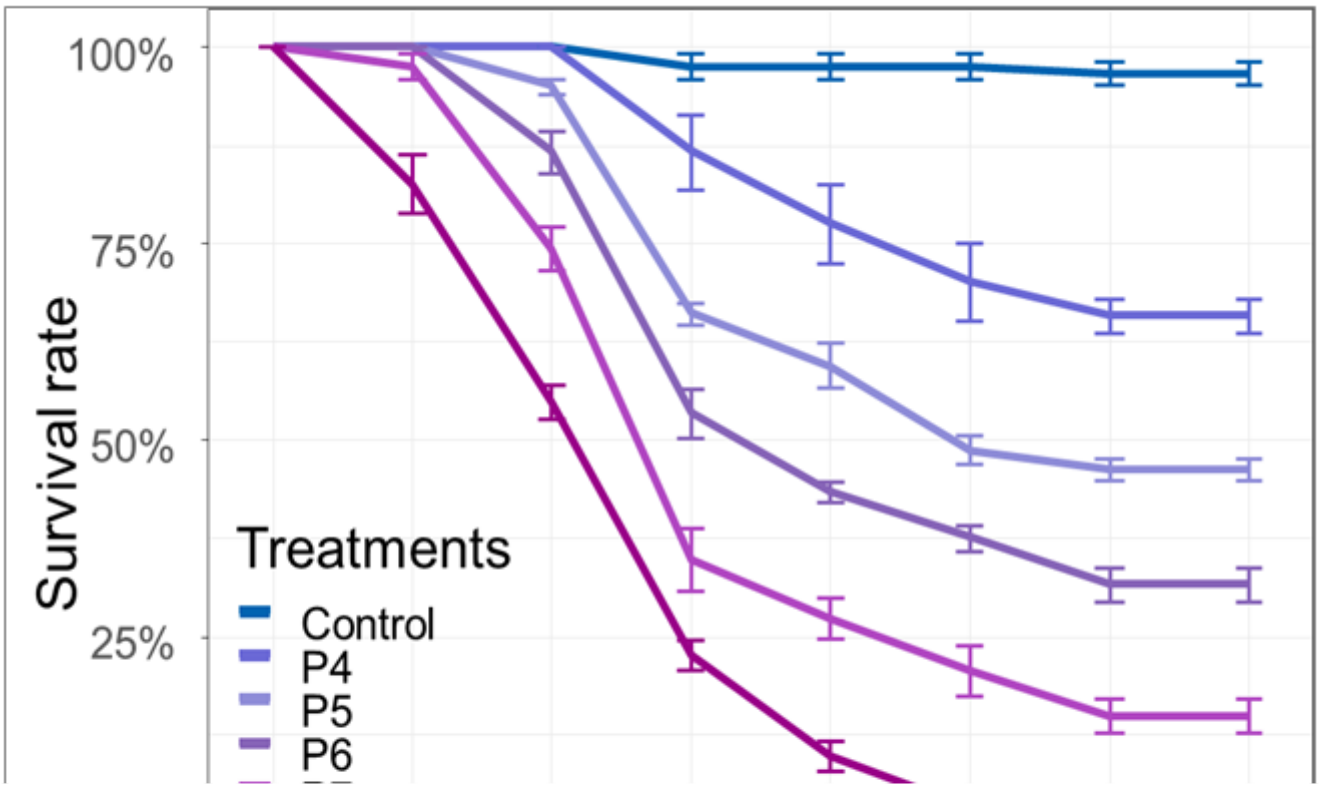

Figure 1

Survival curves of *An. coluzzii* L3 larvae exposed to different concentrations of *C.sp_B*

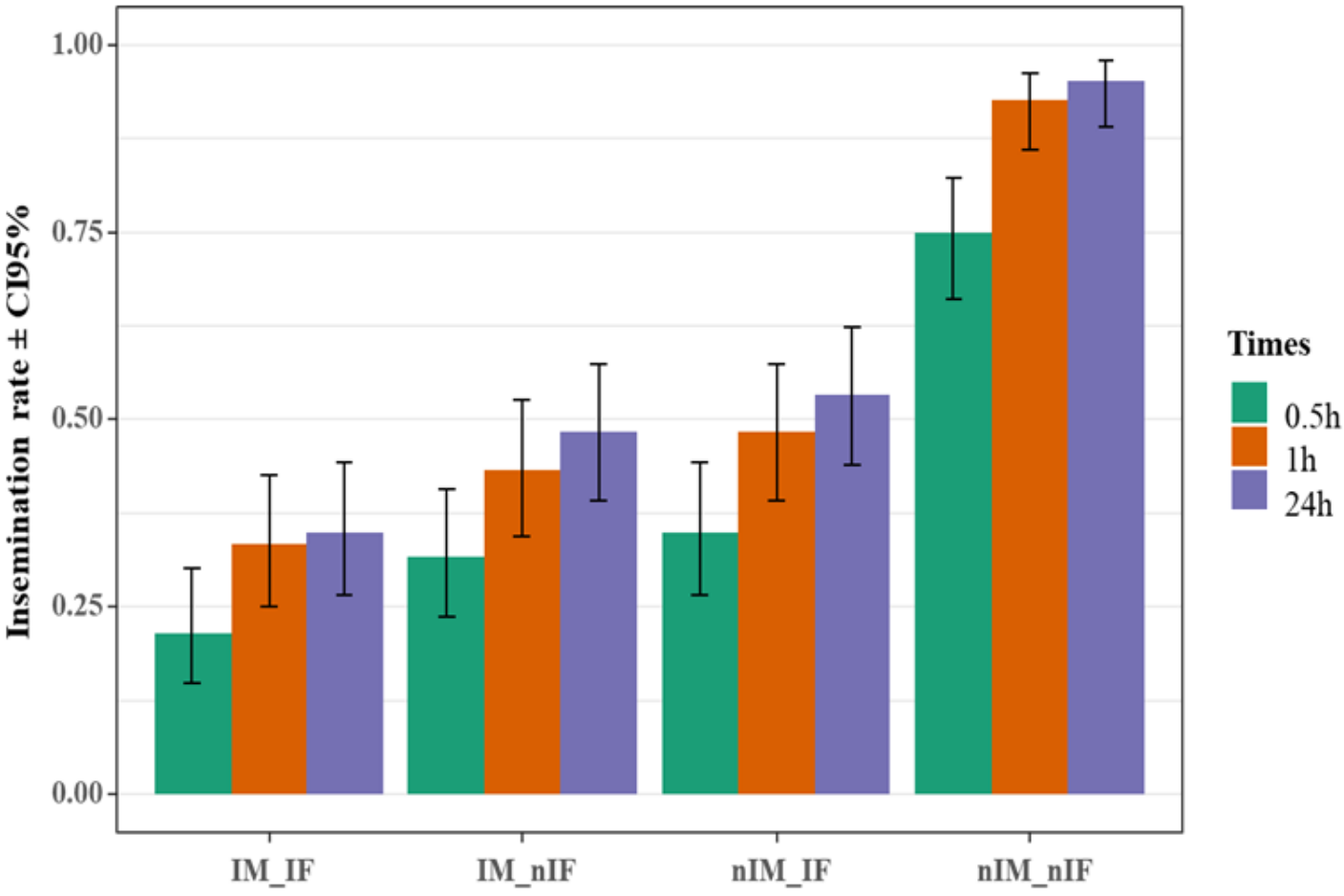

Figure 2

Effects of *C.sp_B infection* on insemination rates of female mosquitoes from different crossing types

Legend: IM= Infected Males; IF= Infected Females; nIM= non-Infected Males and nIF= non-Infected Females

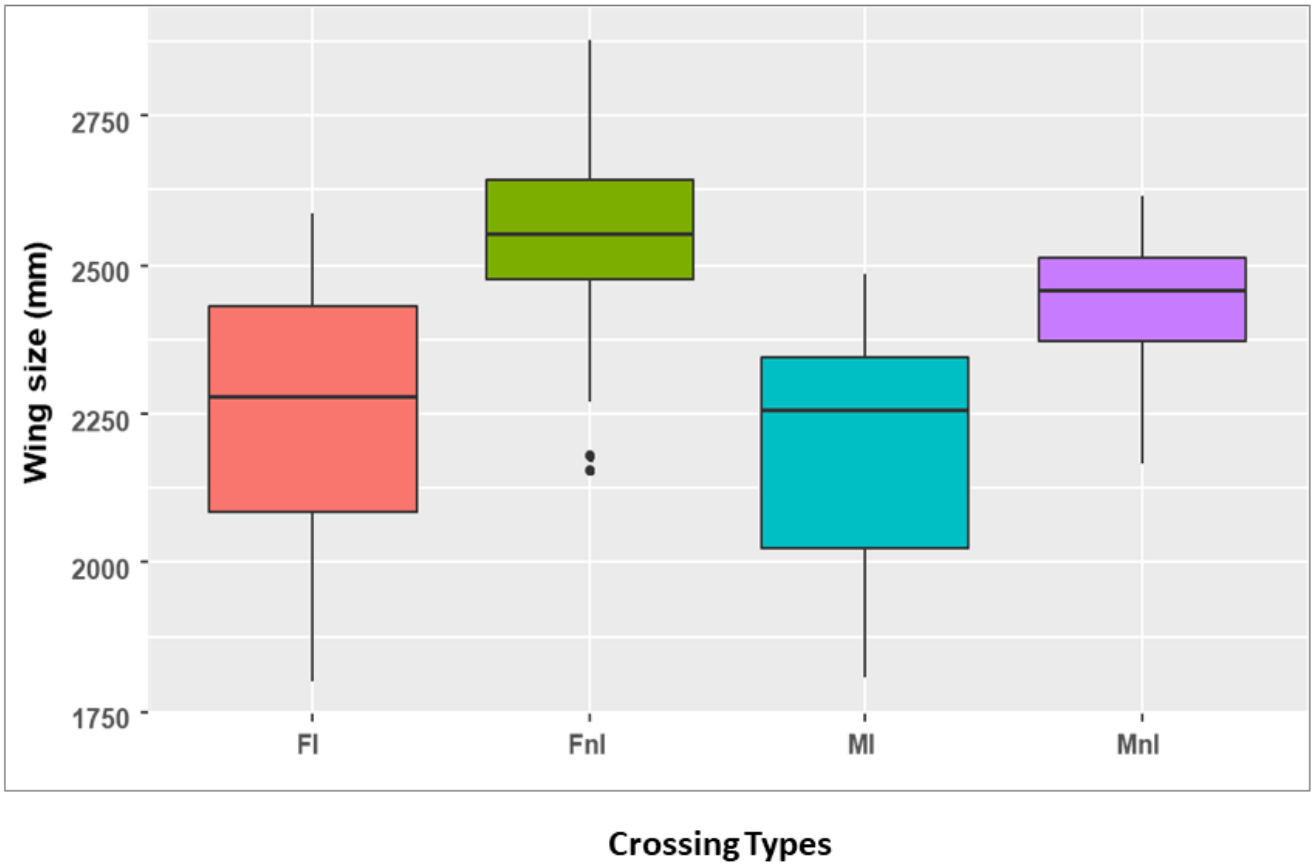

Figure 3

Comparison of wing size between *An. coluzzii* offsprings from *C.sp_B* infected and non-infected mothers

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile1RcodesofAnalysisGnambanetalpdf.pdf