

Investigating Sexual Auto-Dissemination of Native Strain of *Metarhizium pingshaense* from Burkina Faso within *Aedes aegypti* Mosquitoes for Dengue Control Perspectives

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Abstract

In Burkina Faso, recent dengue outbreaks were exacerbated by *Aedes aegypti* mosquitoes, which have developed resistance to conventional insecticides. This study investigates the potential of *Metarhizium pingshaense* Met_S26, a fungus from Burkina Faso, for controlling both endophilic and exophilic *Aedes* mosquitoes. Traditional interventions are less effective against mosquitoes resting outdoors, and the ability of the fungus to be sexually transmitted could enhance its efficacy. *Aedes* mosquitoes were reared and exposed to various concentrations of *Metarhizium pingshaense* to assess its virulence. The fungus showed significant efficacy, with lethal times (LT80) ranging from 8.67 to 11.83 days depending on the concentration. Furthermore, bioassays demonstrated effective sexual auto-dissemination, with substantial transmission of fungal spores between infected and uninfected mosquitoes, leading to lower survival rates in uninfected mates. This study highlights *Metarhizium pingshaense* Met_S26 as a promising tool for controlling both indoor and outdoor *Aedes* populations, complementing existing vector control strategies such as the Sterile Insect Technique (SIT) and *Wolbachia*-based approaches. Future research should explore integrating this fungus with other control methods for a comprehensive vector management strategy.

Keywords

Sexual Dissemination, *Metarhizium pingshaense*, *Aedes aegypti*, Dengue,

1. Introduction

In Burkina Faso, despite considerable efforts to combat infectious diseases, the country experienced significant dengue epidemics in 2016, 2017, and 2023 [1] [2]. The rise of insecticide resistance in the *Aedes aegypti* mosquito has challenged traditional public health measures for controlling the disease [1]. Additionally, mosquito-resting behavior is highly variable, even within the same species, with individuals displaying endophilic (resting indoors) or exophilic (resting outdoors) tendencies after a blood meal [3]. Current interventions targeting indoor mosquitoes, such as long-lasting insecticidal nets and newly developed entomopathogenic fungi, are less effective against exophilic mosquitoes. Their effectiveness may be further threatened by shifts in mosquito behavior toward outdoor resting and feeding [4]. Horizontal sexual transmission of *arhizium* spp. could increase the efficacy of this fungus on both exophilic and endophilic mosquitoes and may complement other promising *Aedes* control strategies, such as the Sterile Insect Technique (SIT) [5], Wolbachia-based technologies [6] and some medicinal plants against *Aedes aegypti* [7]. While previous studies have demonstrated the potential for *Metarhizium* to be sexually transmitted, the inoculum loads (~25 conidia per female mosquito) and the resulting mortality rates have been relatively low [8]. Although auto-dissemination of fungal spores by female *Anopheles gambiae* s.s. has been documented, there is limited data on the use of a more virulent strain, *Metarhizium pingshaense* Met_S26 [9] within *Aedes* mosquitoes, and its ability to transfer from fungus-infected male *Aedes* mosquitoes to uninfected females and vice versa. This study aims to evaluate a new approach for deploying *Metarhizium pingshaense* fungus through sexual auto-dissemination within *Aedes aegypti* mosquitoes, in the context of dengue vector control. It also explores the potential of integrating this strategy with other promising control methods, such as Wolbachia and traditional SIT approaches.

2. Materials and Methods

2.1. *Aedes aegypti* Mosquito Strain

Aedes aegypti were collected as larvae in urban natural breeding sites in Bobo Dioulasso, Burkina Faso, in October 2018. *Aedes* species PCR was carried out and all the strains maintained for building the colony are *Aedes aegypti aegypti*. The larvae were transported to the laboratory of the Institut de Recherche en Sciences de la Santé (IRSS), reared to adults, and blood-fed to generate eggs to start a colony. Adult mosquitoes were kept in the insectary of IRSS-Centre Muraz and maintained at 28°C with 70% - 80% relative humidity and a 12:12-h photoperiod. *Aedes* mosquitoes are maintained in cages (30 cm × 30 cm × 30 cm) and males and females are given 6% Glucose for sugar meal and chicken blood is given to

females for oviposition.

2.2. Preliminary Bioassays of the Virulence of Fungi against *Aedes aegypti* mosquitoes

Bioassays were performed using native strains of *M. pingshaense* isolates *Met_S6* from Burkina Faso [10]. We used an atomizer protocol for infections, as described previously [10]. Three serial concentrations were used: 1×10^8 , 1×10^7 and 1×10^6 conidia/ml. We confirmed that this inoculation technique was able to deliver a repeatable inoculating dose (mean \pm SE): 276 ± 16 spores per mosquito with 1×10^8 , 211 ± 13 spores per mosquito with 1×10^7 spores/ml and 44 ± 3 spores per mosquito with 1×10^6 conidia/ml. Mortality was counted twice daily over two weeks.

2.3. Sexual-Dissemination Test of *Metarhizium pingshaense* Met_S26 Fungi through Sexual Transmission

The nymphs were sorted using a pipette and transferred into a cup containing water. The cups containing the nymphs were then placed inside cages ($30 \times 30 \times 30$ cm) covered with mosquito netting for emergence. The next morning after emergence, at 7:00 am, the male mosquitoes were separated from the females in separate cages. These male and female mosquitoes were fed with 6% glucose soaked in cotton and placed above the mosquito cages. Six types of bioassays were conducted according to Table 1 below to assess self-dissemination through mating. These bioassays were carried out with four biological replicates. Before setting up this experiment, we ensured that the ratio of 25 virgin males to 25

Table 1. Description of different bioassays set-up for sexual auto-dissemination of *Metarhizium pingshaense* Met_S26 within *Aedes aegypti* mosquitoes in mosquito cages.

	B1	B2	B3	B4	B5	B6
Descrip	Twenty-five virgin males and twenty-five virgin females, both infected with fungi were kept together for 48 hours to allow mating. This setup is considered the positive control.	Twenty-five virgin males infected with fungi and twenty-five virgin females not infected with fungi were kept together for 48 hours to allow mating.	Twenty-five virgin females infected with fungi and twenty-five virgin males not infected with fungi were kept together for 48 hours to allow mating.	Twenty-five virgin males infected with fungi and twenty-five virgin males not infected with fungi were kept together for 48 hours. It is assumed that the males do not mate with each other, and any contamination of the non-infected males would result from surface contact.	Twenty-five virgin females infected with fungi and twenty-five virgin females not infected with fungi were kept together for 48 hours. It is assumed that the females do not mate with each other, and any contamination of the non-infected females would result from surface contact.	Twenty-five virgin males and twenty-five virgin females, both not infected with fungi were kept together for 48 hours to allow mating. This setup is considered the negative control.

virgin females over a 48-hour period resulted in more than 95% insemination rates in female mosquitoes. Infected mosquitoes males or females used for bioassays had 211 ± 13 spores per mosquito.

3. Results

The virulence of *M. pingshaense* Met_S26 against females of *Aedes aegypti* mosquitoes over 2 weeks is given in **Figure 1**. The LT80 for the three concentrations of Met_S26 were 8.67 ± 0.17 days, 9.67 ± 0.93 days and 11.83 ± 0.44 day for C1, 1×10^8 conidia/ml; C2, 1×10^7 conidia/ml; C3, 1×10^6 conidia/ml respectively. The LT80 (Lethal Time 80%) is the minimum time required to kill 80% of a population by the action of an insecticide. This sensitivity threshold is set by the World Health Organization for insecticide bioassays [11].

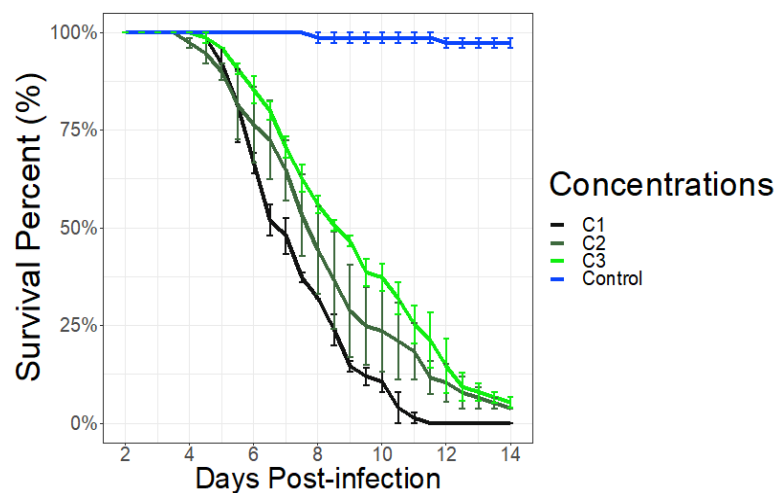


Figure 1. Survival curves of *Ae. aegypti* after exposure to native *Metarhizium pingshaense* Met_S26 from Burkina Faso at three different concentrations: C1, 1×10^8 conidia/ml; C2, 1×10^7 conidia/ml; C3, 1×10^6 conidia/ml. Curves show the percentage survival with error bars indicating 95% confidence intervals from three replicate cages for each line, each containing a starting number of 25 adult females.

As for the sexual dissemination of *Metarhizium pingshaense*, **Figure 2** shows the sexual transmission of the fungus from infected males to uninfected females, and vice versa. This resulted in lower survival rates for uninfected females ($19 \pm 1.9\%$) when mating with fungus-infected males, and survival of $17 \pm 2\%$ for uninfected males when mating with fungus-infected females. All fungus-infected males and females used for the crossing died within 14 days. The hypothesis that uninfected females or males became infected by picking up spores from the surfaces of the mosquito cages where they were confined during cohabitation can be ruled out. During the 48-hour exposure period, both sexes (males and females) walked inside the mosquito cages, which could have facilitated the movement and spread of conidia, leading to passive transmission. However, survival remained similar to that of uninfected control mosquitoes (**Figure 2**).

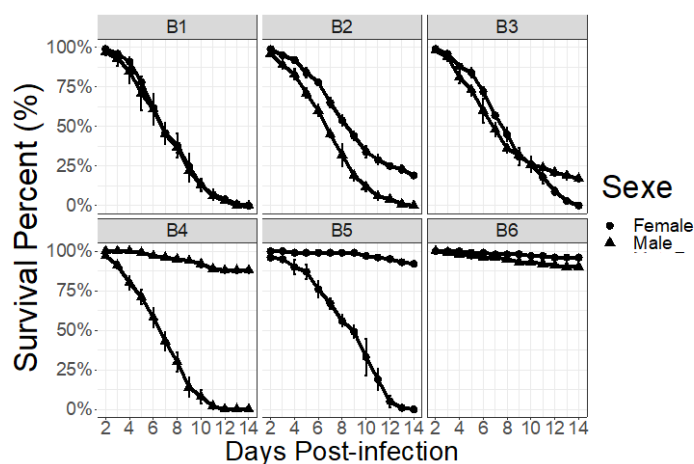


Figure 2. Survival of male and female mosquitoes following 48 hours mating according to different sexual transmission scenarios described in the table 1.

4. Discussion

The LT80 obtained with *M. pingshaense* Met_S26 presents unprecedented and promising results for the safe biological control of *Aedes*-borne diseases. Previous studies have shown that *M. pingshaense* Met_S26 is specific to mosquitoes and does not harm non-target insects such as honeybees and cockroaches [10]. Most fungal-based strategies targeting mosquitoes have been developed for indoor biting species [12]. The high mortality rates observed through sexual auto-dissemination of *Metarhizium pingshaense* suggest a new avenue for controlling outdoor-biting *Aedes* mosquitoes. Information on the auto-dissemination of entomopathogenic fungi via sexual transmission in hematophagous insects is scarce in the literature. The first study addressing this topic was conducted on the tsetse fly, *Glossina morsitans morsitans* Westwood, infected with *Metarhizium anisopliae* and *Beauveria bassiana* in Africa. This study recorded 90% - 100% mortality in both sexes [13]. The second report involved sexual transmission of *M. anisopliae* in *Anopheles gambiae* s.l., the major malaria vector, with auto-dissemination rates of around 30% between males and females [8]. Compared to these earlier works, this current study is the first to demonstrate significantly higher mortality rates resulting from sexual transmission of *Metarhizium pingshaense* in *Aedes aegypti* mosquitoes in the field. This is a critical advancement in mosquito control strategies targeting vector populations in outdoor environments. We hypothesize that *M. pingshaense* Met_S26 could also be integrated with other promising *Aedes* vector control strategies, such as Sterile Insect Technique (SIT) or even as a stand-alone Sexually Transmitted Insecticide (STI). These combinations of strategies could provide a powerful dual approach. However, further investigations are necessary to validate these hypotheses and assess the long-term impact of these methods.

5. Conclusion

This study demonstrated that local strains of *Metarhizium pingshaense* Met_S26

fungus from Burkina Faso exhibit significant virulence against *Aedes* mosquitoes, including the primary vector *Aedes aegypti* that is responsible for transmitting dengue and other arboviruses. Remarkably, the virulence of *Metarhizium pingshaense* extends to sexually transmitted cases within *Aedes aegypti*, underscoring its potential as a novel biological control agent. The findings suggest that *Metarhizium pingshaense* could serve as an effective tool for controlling both indoor and outdoor populations of *Aedes* mosquitoes.

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Authors' Contributions

EB designed the study. EB, FDDH, EJG, SI and SAM performed laboratory and field. EB analysed the data. EB wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Data Availability

Supplementary Materials and Methods, data and codes are available at: <https://github.com/EtienneBilgo/Bilgo-et-al-Advances-in-Entomology>. Correspondence and requests for materials should be addressed to E.B. and A.D.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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