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# Assessment of Local Substrates from Burkina Faso for the Growth of Entomopathogenic Fungi *Metarhizium pingshaense* for Malaria Vector Control Perspectives

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## Abstract

Malaria is a potentially lethal disease caused by parasites of the *Plasmodium* genus, transmitted to humans through the bite of infected female mosquitoes, primarily *Anopheles*. To control this disease, various strategies are implemented, including biological control, which targets the vectors of the parasite. This approach uses biological agents such as entomopathogenic fungi, including *Metarhizium pingshaense*, a fungus capable of causing lethal infections in mosquitoes. The production of *Metarhizium pingshaense* is still limited in Burkina Faso, and local cultivation of this fungus could help fill this gap. A study was conducted to identify optimal local substrates that promote its growth. Indeed, after gathering information on the dietary habits of populations in Bobo-Dioulasso and Bama, three potential substrates were selected: rice, cornmeal dough (MFL), and beans. These substrates were inoculated with two strains of *Metarhizium pingshaense* (S10 and S26) to assess their ability to support fungal growth and their effectiveness. Experimental results showed that MFL and bean substrates favored optimal growth of *Metarhizium pingshaense*, with growths of 1.91 cm and 2.13 cm after 8 days, compared to 1.83 cm on a standard media (PDA). In terms of virulence, S26 strain caused 60% mosquito mortality on both the bean and PDA media, while S10 strain induced mortalities of 50% for bean and 62% for PDA.

**Keywords**

Malaria, *Anopheles*, Biological Control, *Metarhizium pingshaense*, Local Culture Media, Burkina Faso

## 1. Introduction

Malaria, the most common parasitic infection, remains a major global health issue. According to estimates by the World Health Organization [1], approximately 263 million malaria cases were recorded in 2023, with 597,000 associated deaths, representing an increase of 11 million cases compared to 2022 [1]. About 95% of cases are concentrated in 29 countries, primarily in sub-Saharan Africa. In Burkina Faso, the estimated number of cases is around 8.1 million, with over 16,146 deaths in 2023 [2]. Malaria is caused by *Plasmodium* parasites, transmitted to humans by the bite of infected female *Anopheles* mosquitoes [3]. The control of malaria relies on two main approaches: the use of antimalarial treatments to eradicate the parasites in humans and vector control through insecticides [4]. However, the growing resistance of mosquitoes to insecticides and parasites to antimalarial drugs has significantly reduced the effectiveness of these methods [5].

In response to this situation, alternatives such as biological control using microorganisms are being actively explored [6]. Among these microorganisms, entomopathogenic fungi are of particular interest due to their ability to regulate insect populations [7]. Unlike chemical insecticides, which persist in the environment for a long time, these fungi remain for a shorter duration, limiting their negative effects on ecosystems [8]. One particularly promising entomopathogenic fungus is *Metarhizium pingshaense*, a recently identified a strain with high virulence against mosquitoes isolated in Burkina Faso [9]. Indeed, this strain have shown remarkable efficacy in killing mosquitoes and adapting to local environmental conditions. This specificity makes it a more suitable alternative to foreign strains, as it could offer better effectiveness and sustainability in the fight against malaria vectors. *Metarhizium* hold a significant promise for biological insect control due to their potential for low-cost, large-scale local production, with many strains already available commercially. For instance, *Beauveria bassiana and Metarhizium anisopliae* has been successfully cultivated on various solid substrates [10], cheap media [11], and steamed rice [12]. More than 14 companies produce strains of *Beauveria* (e.g., *B. bassiana* and *B. brongniartii*), while over 10 companies, including some based in Africa, manufacture *Metarhizium* species (such as *M. anisopliae* and *M. anisopliae var. acridum*), targeting a wide range of insect [13].

However, industrial production of *Metarhizium pingshaense* is nearly nonexistent in Burkina Faso, limiting its use on a large scale. To address this gap, it is crucial to develop local cultivation methods for the fungus. In this way, one of the main challenges is identifying suitable local substrates capable of supporting fungal growth and optimizing production at the community level. Therefore, the

objective of this study was to evaluate the effectiveness of various local substrates for the indigenous cultivation of *Metarhizium pingshaense*. Specifically, the study aimed to: i) identify potential local substrates, ii) assess their effectiveness in terms of fungal growth and virulence, and iii) determine the correlation between fungal virulence and the characteristics of the substrates used.

## 2. Methodology

**Survey on the dietary habits of local populations**

The survey was conducted in households in the cities of Bobo-Dioulasso and Bama to gather information on the dietary habits of the local populations. It involved 15 randomly selected households per collection site to ensure a diverse representation of local food practices. The main objective of the survey was to identify commonly consumed foods, to select the most suitable local substrates for cultivating *Metarhizium*. The sampling included households from various socio-economic groups to ensure a representative coverage of the population. This approach helped identify the most frequently used foods in the region that could be used to create effective and accessible culture media.

**Preparation of media**

**Preparation of rice:** 200 ml of water was boiled in a pot, and 100 g of rice was added and cooked on low heat for at least 30 minutes. After cooking, the preparation was distributed into petri dishes under a laminar flow hood.

**Preparation (MFL):** Two types of maize dough were prepared: one with tamarind and one without.

**Without tamarind:** $\frac{1}{2}$ liter of water was boiled, and 100 g of maize flour was diluted in 150 ml of warm water, then added to the boiling water in small portions while stirring with a wooden spatula. The mixture was covered and simmered for 20 minutes, then distributed into petri dishes under a laminar flow hood.

**With Tamarind:** After adding the diluted flour to the boiling water, tamarind juice was added, and the mixture was stirred and simmered for 20 minutes, then distributed into petri dishes under the hood.

**Preparation of beans:** 100 g of beans were boiled in 1 L of water for 2 hours. After cooking, the beans were placed in petri dishes under the hood.

**Preparation of *metarhizium* suspensions and inoculation on various media**

The laboratory already had *Metarhizium pingshaense* strains S10 and S26 on PDA medium. For each solution, samples of the *Metarhizium pingshaense* strains were scraped using an inoculation loop and added to a 0.05% Tween 80 solution or distilled water in Eppendorf tubes. The final concentration used in the assays was 1 × 107. These tubes were vortexed for several minutes to ensure a homogeneous solution. Under the hood, the solutions were pipetted into petri dishes containing the various culture media (rice, MFL without tamarind, MFL with tamarind, beans, and the PDA control) and inoculated. The petri dishes were then sealed with parafilm to prevent contamination and placed in an incubator at 27˚C for 8 days to allow fungal growth.

**Culture observations**

**Macroscopic observation**: The petri dishes were observed daily during 8 days to monitor growth fungal colonies.

**Microscopic observation**: The fungi were examined using morphological criteria to classify *Metarhizium* species, as described by [14]. Fungal samples from the various media were mixed with Tween 80 or distilled water and vortexed for several minutes to create homogeneous solutions. These were placed between slides for optical microscopy observations at 40x magnification to observe the morphology of the spore.

**Mosquitoes rearing**

*Anopheles coluzzii* were reared in the laboratory at the Institut de Recherche en Sciences de la Santé (IRSS) in Bobo Dioulasso, Burkina Faso, under standard conditions. The temperature was maintained at 27 ± 2˚C and the relative humidity was set at 70 ± 5% with the rooms with photoperiod of 12L:12D. Larvae were kept in plastic trays filled with tap water. Larvae from stage L1 to stage L4 were fed with Tetra-min® (Tetra, Melle, Germany). The resulting pupae were transferred to cages of size (30 × 30 × 30 cm), where they emerged as adults with access to 6% glucose.

**Mosquitoes' infection method using *Met_S*10 and *Met_S*26**

A total of 150 female *Anopheles coluzzii* mosquitoes were divided into six groups of 25 mosquitoes each. The mosquito infection was performed by spraying mosquitoe body with fungal suspension. After that, the mosquitoes were then transferred to cardboard cups and kept in the laboratory until they regained flight. Once their ability to fly was restored, they were monitored for mortality over 14 days, dead mosquitoes being counted and removed twice daily.

**Determining the cause of mortality**

The cuticle of each dead mosquito was disinfected with 1% bleach for 20 seconds to remove residual spores from the surface, as the spores responsible for killing the mosquito would have penetrated its cuticle. After rinsing with sterile distilled water, the mosquitoes were placed on agar (1%) to allow fungal hyphae growth. After five days of incubation, the presence of hyphae indicated that the fungus had caused the mosquito's death.

**Data processing and statistical analysis**

Data was entered and verified using Microsoft Excel 2016. Potential local substrates data were analyzed using the graphical functions of Excel 2016. Statistical analysis was performed with R software. Fungal growth rates and virulence were assessed using the ggplot function in R. A one-way ANOVA was used to compare means, with a significance threshold set at 0.05.

## 3. Results

**Potential local substrates**

The survey results, as shown in **Figure 1**, revealed that foods such as rice (33.33%), MFL (33.33%), beans (13.33%), pasta (10%), fonio (4,44%) couscous, casava couscous, and yam (each 1.11%), as well as ground peas (2.22%), were

frequently consumed in the households.

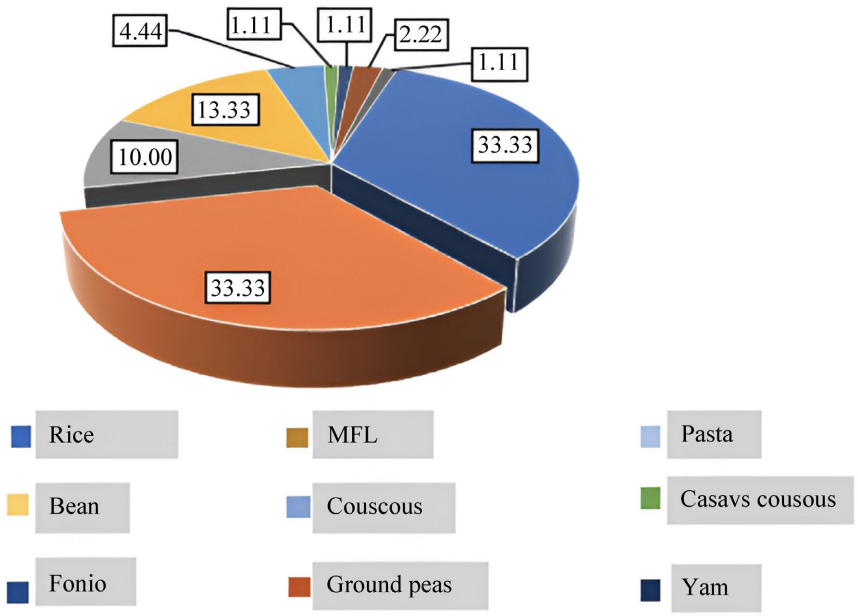


**Figure 1.** Frequency of food consumed in households.

### Growth rate

After collecting data on the culinary habits of the local population, the selected foods were used to prepare culture media, on which we inoculated our fungi and monitored their growth rate. Two local media were of particular interest during the experiment. As shown in **Figure 2**, the bean-based medium was the most favorable for fungal development, with the fungi covering a diameter of 2.13 cm in 8 days. The MFL without tamarind based medium also allowed good growth of *Met.*, with a coverage reaching 1.9 cm in diameter over the same period. Unfortunately, we did not observe any fungal growth with the MFL containing tamarind. These two media therefore facilitated better fungal growth compared to the standard PDA medium, commonly used in the laboratory, which resulted in a growth diameter of 1.83 cm.

### Virulence of fungal infections in female *Anopheles* mosquitoes

From the previous study, the bean-based medium was found to be the most favorable for fungal growth (S10 and S26). We therefore sought to test the entomopathogenic capacity of these fungi, which developed rapidly on this medium. The results of this test are shown in **Figure 3**, where the virulence of the fungi produced on the bean medium was compared to those produced on our standard medium, PDA, typically used for fungal production. After 10 days of infection, the S26 strain produced on the bean medium had already matched the virulence of the strain produced on the PDA medium, both having killed 60% of the mosquito population in 10 days. On the other hand, for the S10 strain, the PDA medium produced more virulent fungal spores, killing 62% of the mosquitoes, thus outperforming the S10 strain produced on the bean medium, which eliminated

50% of the mosquitoes.

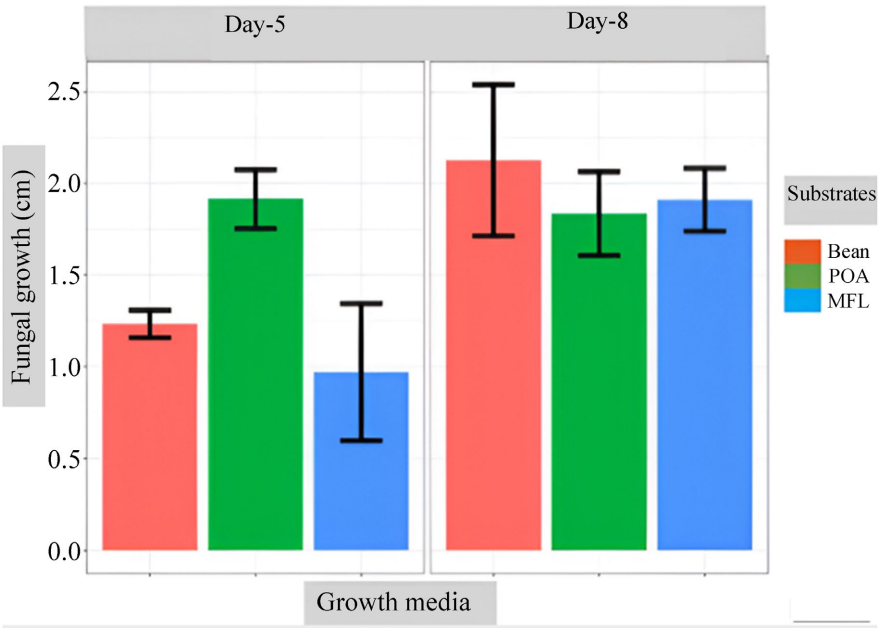


**Figure 2.** Growth rate of *Metarhizium pingshaense* on local and PDA culture media.

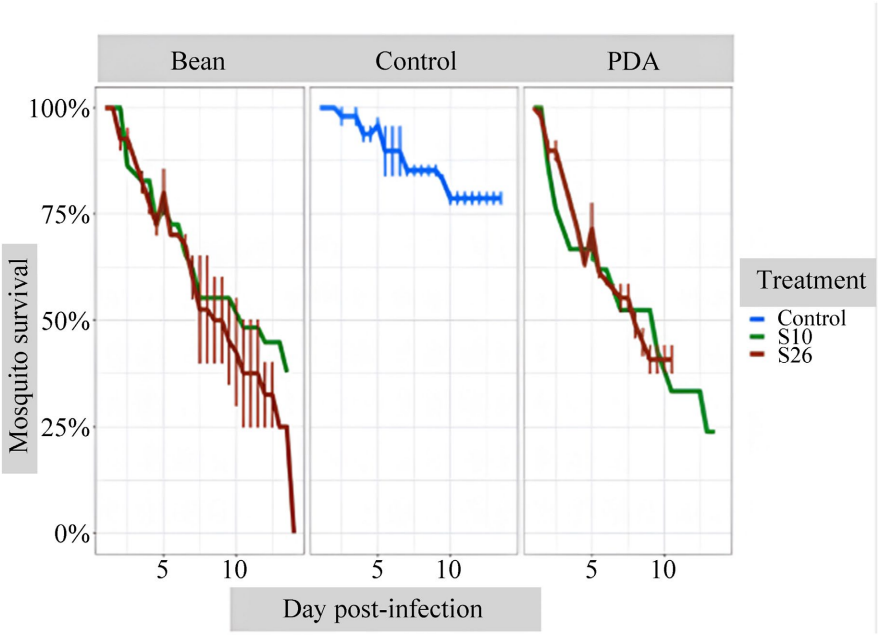


**Figure 3.** Virulence of *Metarhizium* strains as a function of culture media.

## 4. Discussion

The present study investigates the cultivation of *Met.* on local substrates commonly consumed by the Burkina population, based on surveys and experiments. An analysis of local culinary habits revealed that rice, MFL (a traditional dish made from millet or maize), and beans are the most consumed foods in the site

were we conducted the study in Burkina Faso, likely due to their availability and affordability. An alimentary survey conducted between 2013 and 2019 confirmed that these foods were the staples of the population [15]. Based on these findings, the study proceeded to cultivate *Metarhizium pingshaense* on local culture media prepared from these foods to observe fungal growth.

After 8 days of incubation, two substrates stood out: tô and beans. On these substrates, fungal biomass production was significantly higher than on the standard PDA (Potato Dextrose Agar) medium [16]. Tô and bean-based media were found to contain favorable components for fungal growth, including proteins, carbohydrates, lipids, and dietary fibers [15]. In contrast, rice-based media, though rich in proteins and carbohydrates, showed poor fungal development due to its low fiber and lipid content. This suggests that the fungi thrived better on maize (tô), beans, and potatoes, which contain higher levels of dietary fiber, compared to rice. Beans, being particularly rich in dietary fiber, outperformed tô, and thus the bean-based medium produced the highest fungal biomass.

However, previous studies reported good fungal growth on rice-based media due to its starch content [17], which contrasts with our findings. The discrepancy might be explained by differences in the quality of rice used, with the rice in our study potentially having lower starch content [15]. Additionally, the fungal species used in our study, *Metarhizium pingshaense*, may prefer to metabolize dietary fibers rather than starch, unlike *Metarhizium anisopliae* used in earlier studies [18]. The two species are distinguishable primarily through molecular analysis [9], but macroscopic differences in their ability to metabolize starch and fiber could explain the varying results. *Metarhizium pingshaense* may be better suited to break down cellulose, a major component of dietary fiber, as it has been identified in plant rhizospheres in Burkina Faso [9] [19] [20].

Regarding production time, the PDA medium outperformed the others, yielding more biomass after 5 days. This is likely because the fungi had already adapted to PDA, whereas they required more time to adjust to new media like MFL and bean-based substrates.

The study also assessed the virulence of *Metarhizium* fungi grown on local media against mosquitoes. The bean-based medium, which produced the highest fungal biomass, was chosen for virulence testing and compared to the PDA medium. The results showed that the *Metarhizium* S26 isolate grown on the bean-based medium killed 60% of mosquitoes after 10 days, similar to the performance of fungi grown on PDA. This suggests that the bean-based medium could be used as an alternative for fungal production, maintaining the fungi's entomopathogenic effectiveness. However, the *Metarhizium* S10 isolate from the bean-based medium showed reduced virulence compared to the PDA-grown fungi, killing only 50% of mosquitoes over the same period, while the PDA-grown fungi killed 62%. Despite this, the mortality rates followed similar trends from day 1 to day 9, with a marked deviation only between days 9 and 10, which may be due to a clustered mortality event among mosquitoes infected with *Metarhizium* S10 from the

PDA medium. This discrepancy could be resolved with further observation and research, and does not entirely rule out the possibility that the bean-based medium may slightly reduce the virulence of *Metarhizium* S10 strains.

## 5. Conclusion

This study highlights the potential of using local substrates such as beans and maize dough for the sustainable and effective cultivation of *Metarhizium pingshaense* for malaria vector control. The use of these locally available materials could not only improve the effectiveness of biological control but also provide a cost-effective method for large-scale production of the fungus in malaria-endemic regions, particularly in Burkina Faso. Further studies should focus on optimizing fungal production methods and assessing their long-term efficacy in the field.

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## Author Contributions

EB, and IS conceived, co-ordinated and supervised the study. S.I. and CLT. EB. analysed the data and S.I, CT, EB, AJL and AD wrote the manuscript. All authors reviewed, edited and approved the final version of the manuscript.

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## Availability of Data and Materials

All data for this study will be available upon request.

## Conflicts of Interest

The authors declare no competing interests.

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## Abbreviations

| | |
|---|---|
| Met. | *Metarhizium pingshaense* |
| MFL | Cornmeal dough |
| PDA | Potato Dextro Agar |
| S10 | *Metarhizium pingshaense* strain recorded as number 10 in the fungal strain collections |
| S26 | *Metarhizium pingshaense* strain recorded as number 26 in the fungal strain collections |