

Mortality Reduction and Food Consumption of Fall Armyworms *Spodoptera frugiperda* and Non-Target Insect *Cirina butyrospermi* after Exposure to Burkinabe Native *Entomopathogenic fungi Metarhizium* sp.

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How to cite this paper: Bilgo, E., François, H.D., Saré, I. and Diabaté, A. (2025) Mortality Reduction and Food Consumption of Fall Armyworms *Spodoptera frugiperda* and Non-Target Insect *Cirina butyrospermi* after Exposure to Burkinabe Native *Entomopathogenic fungi Metarhizium* sp. *Advances in Entomology*, 13, 68-74.

<https://doi.org/10.4236/ae.2025.131004>

Received: September 23, 2024

Accepted: November 25, 2024

Published: November 28, 2024

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Abstract

Fall Armyworms (FAW) *Spodoptera frugiperda*, particularly in maize crops, is a major agricultural pest. Current control methods rely heavily on chemical pesticides, which are costly and harmful to non-target organisms. To provide a sustainable alternative, the research isolated indigenous *Entomopathogenic fungi*, *Metarhizium* sp. and *Trichoderma* sp., from FAW-infested areas. The fungi were tested for virulence against FAW and a non-target insect, *Cirina butyrospermi*. Results showed that *Metarhizium* sp. FAW was highly effective in killing FAW while sparing *Cirina butyrospermi*, demonstrating its potential as a biological control agent. In addition, FAW infected with *Metarhizium* sp. exhibited a significant reduction in food consumption. The study highlights the potential use of indigenous fungi in integrated pest management systems, reducing the reliance on chemical pesticides and supporting sustainable agriculture. Further research is needed to understand the interaction of these promising *Metarhizium* strains with soil microbiota and their long-term efficacy in field conditions.

Keywords

Fall Armyworms, Burkina Faso, *Metarhizium*, Biocontrol, *Entomopathogenic fungi*

1. Introduction

This template, created in MS Word 2007, provides authors with most of the formatting. The emergence and rapid spread of the Fall Armyworm (FAW) *Spodoptera frugiperda* in Africa pose significant threats to the food and income security of millions of smallholder farmers [1]. FAW is now confirmed in over 30 countries and is likely to become endemic in many sub-Saharan African nations [1]. According to the latest United Nations report on world food security and nutrition, hunger has been rising globally since 2016, affecting more than 815 million people, including 243 million in Africa [2]. In Burkina Faso, food security and nutrition have been particularly jeopardized by FAW infestations, especially in maize crops, leading to thousands of people facing extreme hunger in the past two years [3]. During the 2018 rainy season, statistics from the Burkina Faso Ministry of Agriculture indicated that, as of July 29, only 3 out of 13 administrative regions were spared from armyworm infestations; the eastern region alone reported 5,000 hectares of infested land [4]. Control of FAW primarily relies on chemical pesticide applications, which raises multiple concerns, including the rapid development of insecticide resistance [5], the toxicity of these chemicals to non-target organisms and ecosystems [6], and their prohibitive costs for smallholder farmers [7]. In response, agricultural biotechnologies are emerging as viable alternatives for sustainable pest management. Innovative management practices, such as the use of *Entomopathogenic fungi*, may offer effective and eco-friendly solutions to control this invasive pest in Africa [8]. In this study, we isolated indigenous *Entomopathogenic fungi*, specifically *Metarhizium* sp. FAW. and *Trichoderma* sp., from field collections of FAW. We directly tested the efficacy of these fungi against FAW and selected non-target insects, assessing their potential as biological control agents. Additionally, we evaluated the impact of fungal infection on insect feeding behavior and discussed the future application of these fungi in field settings against FAW.

2. Materials and Method

2.1. Collection of Fall Armyworms for Fungal Isolation

A total of 67 Fall Armyworm (FAW) larvae were randomly collected from 12 infested maize farms over four consecutive days from July 22 to 26, 2018, in Soumouso (11°04'N, 4°03'W), Western Burkina Faso. The larvae were brought to the laboratory and kept in individual plastic dishes, provided with maize leaves and lithium as food until their death.

2.2. Isolation and Identification of Local Fungal Strains from Fall Armyworm in Burkina Faso

Dead larvae were individually washed with bleach, rinsed in sterile water, and placed on agar plates for five days to observe for mycosis. Only 11 larvae exhibited signs of mycosis. These larvae were then crushed and plated on a fungal selective medium composed of 42 g of potato-dextrose agar, 0.5 g of chloramphenicol, and

0.6 g of cetyl trimethylammonium bromide per liter. Fungal isolates from the larvae were identified using macro-morphological characteristics, including conidogenesis, radial growth estimation, spore color, and mycelial texture on PDA media, following the methods outlined by Wyrebek *et al.* Additionally, we utilized microscopic morphology to identify *Metarhizium* spp. spores as described by Wyrebek *et al.* (2011) [9]. The pure colonies from dead larvae of FAW, which was characterized as *Metarhizium* sp. and was confirmed through genus PCR by amplifying the TIS region were used for bioassays. We named this strain *Metarhizium* FAW.

2.3. Virulence and Food Consumption Assays of *Metarhizium* spp. against Fall Armyworm and Non-Target Insect *Cirina butyrospermi*

Virulence assays were conducted on Fall Armyworms (FAW) and the non-target, edible larva of the butterfly *Cirina butyrospermi* Vuillet, locally known as “shitumu” in the Burkina Faso national Dioula language, from Soumouso. The assays tested the effectiveness of *Metarhizium* sp. strains isolated from FAW, alongside *Metarhizium pingshaense*, a mosquitocidal strain, and *Trichoderma* sp. FAW. Strains isolated from maize rhizosphere. For all three treatments: *Metarhizium* FAW, *Metarhizium pingshaense*, and *Trichoderma* sp. FAW larvae were sprayed with three concentrations: C1 = 1×10^8 conidia/mL, C2 = 1×10^7 conidia/mL, and C3 = 1×10^6 conidia/mL, following the protocol described by Bilgo *et al.*, 2018. For comparison, Deltamethrin at 0.05%, a commonly used pyrethroid in chemical FAW control programs, was used as a chemical control. Additionally, a negative control, consisting of a blank solution of 0.05% Tween without any fungi, was applied to both FAW and *Cirina butyrospermi* larvae. Insect mortality was monitored over a two-week period, and food consumption was indirectly measured by quantifying the waste produced by the insects when giving leaves of maize and shea to FAW and larva of the butterfly *Cirina butyrospermi* respectively. All bioassays were performed with five biological replicates per treatment.

3. Results and Discussion

3.1. Survival of Fall Armyworms *Spodoptera frugiperda* and Non-Target Insect *Cirina butyrospermi* after Exposure to Burkinabe Native *Entomopathogenic fungi* and Chemical Insecticides

Figure 1 shows the reduced survival of Fall Armyworms (FAW) infected with varying concentrations of *Metarhizium* sp. FAW, originally isolated from FAW. The highest concentration (C3 = 1×10^8 conidia/mL) killed 100% of FAW within 8 days. The other two concentrations also achieved 100% mortality within two weeks of exposure. In contrast, FAW exposed to other treatments, such as *Trichoderma* sp. FAW and *Metarhizium pingshaense* (even at the highest concentration of C1 = 1×10^8 conidia/mL), exhibited higher survival rates of $2 \pm 1.67\%$ and 41

$\pm 4.17\%$, respectively, compared to *Metarhizium* sp. FAW. Interestingly, Deltamethrin, a widely used chemical for FAW control, resulted in a survival rate of $\sim 19 \pm 4.75\%$, indicating potential resistance development to this insecticide (**Figure 1**). For the non-target insect *Cirina butyrospermi*, exposure to *Metarhizium* sp. FAW showed similarly low survival rates ($\sim 3\%$) at the highest concentration ($C1 = 1 \times 10^8$ conidia/mL), comparable to the effect of *Trichoderma* sp. FAW. However, at this concentration, *Metarhizium pingshaense* exhibited a higher survival rate ($\sim 48\%$) for *Cirina butyrospermi* (**Figure 1**). Furthermore, FAW exposed to *Metarhizium* sp. FAW at lower concentrations ($C1 = 1 \times 10^7$ conidia/mL and $C1 = 1 \times 10^6$ conidia/mL) demonstrated higher survival rates, similar to those seen with *Trichoderma* sp. FAW and *Metarhizium pingshaense* (**Figure 1**). These virulence data suggest that *Metarhizium* sp. FAW is highly specific and virulent to FAW while sparing the non-target insect *Cirina butyrospermi*. Genomic studies are currently underway to determine the relationship between this strain and the well-known *Metarhizium rileyi*, another FAW-specific strain [10] [11]. Future semi-field and field studies will be conducted to further assess the efficacy of *Metarhizium* sp. FAW in controlling FAW populations. Environmental factors such as temperature, humidity, water availability, and solar radiation may influence the germination, growth, and viability of *Entomopathogenic fungi* in the field [12].

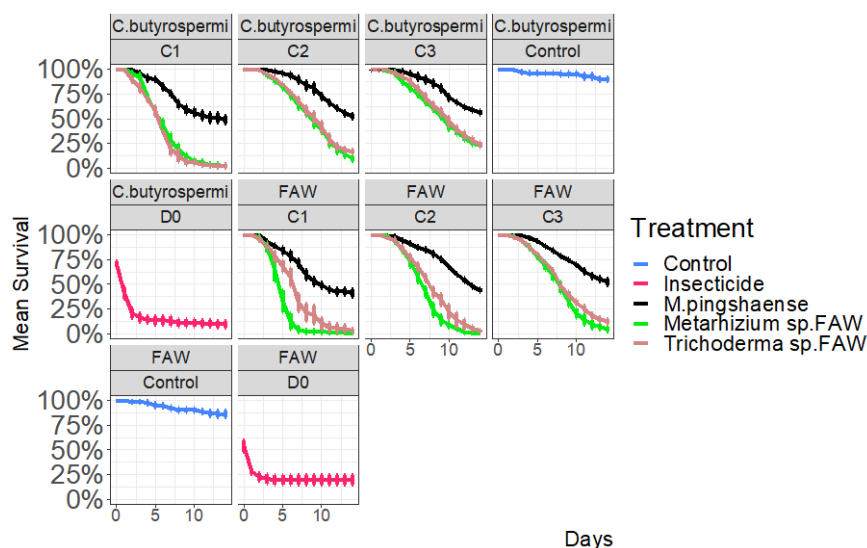


Figure 1. Survival curves of Fall Armyworms *Spodoptera frugiperda* and non-target insect *Cirina butyrospermi* after exposure to Burkinabe native *Entomopathogenic fungi* and insecticides. Concentrations: $C1 = 1 \times 10^8$ conidia/ml, $C2 = 1 \times 10^7$ conidia/ml, and $C3 = 1 \times 10^6$ Conidia/ml.

3.2. Impact of Fungal Infections on FAW Food Consumption

Figure 2 illustrates the reduction in food consumption in FAW after exposure to *Metarhizium* sp. FAW. FAW exposed to the highest concentration ($C1 = 1 \times 10^8$ conidia/mL) of *Metarhizium* sp. FAW ceased feeding on maize leaves within five days post-infection. This reduction in feeding was significantly greater than that

observed with other fungal strains or insecticides. Importantly, *Metarhizium* sp. FAW did not affect the food consumption of the non-target insect *Cirina butyrospermi*, with results similar to those observed with *Metarhizium pingshaense* and *Trichoderma* sp. FAW. This pre-lethal reduction in food consumption is a key advantage of using *Metarhizium* sp. FAW as a biological control agent over chemical insecticides. Future studies should focus on understanding the interactions between *Metarhizium* sp. FAW and other soil microbiota, as well as its long-term efficacy in pest management under field conditions. Integrating this biocontrol agent into an Integrated Pest Management (IPM) framework could provide smallholder farmers in Burkina Faso and across sub-Saharan Africa with an effective, low-cost strategy for controlling FAW while reducing reliance on chemical insecticides.

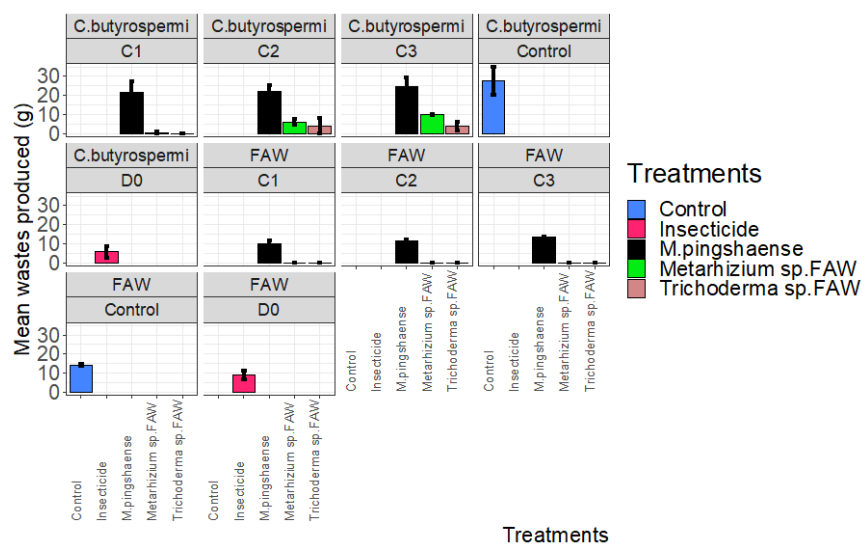


Figure 2. Food consumption reduction of Fall Armyworms *Spodoptera frugiperda* and non-target insect *Cirina butyrospermi* after exposure to Burkinabe native *Entomopathogenic fungi* and insecticides. Concentrations: C1 = 1×10^8 conidia/ml, C2 = 1×10^7 conidia/ml, and C3 = 1×10^6 Conidia/ml.

Future studies should also focus on understanding the interactions between *Metarhizium* sp. FAW and other soil microbiota, as well as its long-term efficacy in pest management under field conditions. Integrating this biocontrol agent into an integrated pest management (IPM) framework could provide smallholder farmers in Burkina Faso and across sub-Saharan Africa with an effective, low-cost strategy for controlling FAW and reducing reliance on chemical insecticides.

4. Conclusion

This study establishes a promising foundation for the isolation of local *Metarhizium* strains for use in the biological control of Fall Armyworm (FAW). The successful identification of indigenous fungal strains lays the groundwork for future biocontrol projects in Burkina Faso aimed at combating this emerging pest.

However, further research is essential. Laboratory and field assays should be conducted to assess whether this strain of *Metarhizium* can be artificially established as an endophyte in maize roots and leaves. Moreover, investigations should explore the effects of endophytic *Metarhizium* on maize growth, pest resistance, and overall crop health. Evaluating the potential of *Metarhizium* to form symbiotic relationships with maize could significantly enhance its application in sustainable agricultural practices, offering an eco-friendly alternative to chemical pesticides.

Acknowledgements

We are very grateful to Mr. Olivier Zida for collecting Fall Armyworm samples used in this study and for providing technical assistance.

Authors' Contributions

EB and AD designed the study. EB, FDDH and IS 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/Advances-In-Entomology_Bilgo-et-FAW

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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