

Response to the recommender and reviewers

Revision round #1

Decision for round #1 : *Revision needed*

Recommender Kévin Tougeron

As the authors will see from the reviews by two experts in the field, the manuscript has many merits and could be recommended by PCI Zoology, provided several clarifications are made.

I would draw the authors' attention to the need to place the results of this study in a more general context, to see what could be extrapolated from the analyses, for example on other systems.

Answer: *First of all, we would like to thank Mr Tougeron for considering our article for recommendation by PCI and we thank him and the reviewers for the time they spent reviewing our manuscript. We considered all the points raised and have carefully and thoroughly revised the manuscript.*

*Following reviewer comments, we propose a new title: "Dose, temperature and formulation shape *Metarhizium anisopliae* virulence against the oriental fruit fly: lessons for improving on-target control strategies".*

A section was added at the end of the abstract and at the end of the discussion to generalize.

by Kévin Tougeron, 06 May 2024 10:42

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version: 1

Review by François Verheggen

Metarhizium anisopliae is known for its potential as a biological control agent of the oriental fruit fly, *Bactrocera dorsalis*. Chailleux and colleagues have performed an interesting study demonstrating the importance of the inoculation dose, the presence of adjuvant in the formulation and the air temperature on *Metarhizium* virulence. Given the importance of the fruit fly species and the need for identifying efficient control strategies, I believe this manuscript is of prime importance.

Moreover, the study has been properly designed and executed. Below a just minor comments, that should be taken into account to increase clarity. Authors should pay special attention to the incomplete methodology section regarding the role of the adjuvant.

Material and Method:

"As this design provided high mortality even for the smallest dose, we modified our inoculation method to be able to reduce the spore load on flies. To this end, we used a paint brush with a small number of hairs (8, 4, 2, and 1 hair)"

=> It is not clear to me what has been modified in the inoculation method, and what is the role of the paint brush.

Answer: *This was complemented. The paintbrush was used to manually apply the spores onto the flies.*

"Among the 30 flies of each type"

=> Do you mean of each sex?

Answer: Yes, this was changed.

" The same inoculation procedure of flies as described above was adopted but only with the dose of 40 084 926 [spores.cm⁻²](#). "

=> I believe you should justify this choice.

Answer: This has been added. This choice was made in the light of previous results because, from an applied perspective, we targeted a dose that would kill the flies but not too quickly to allow transmission to conspecifics.

"The quantity of spores in the inoculation tube was kept constant. Tests were led"

=> The methodology section on the evaluation of the role of the adjuvant on fly mortality if incomplete.

Answer: In fact, the sentence should have been deleted entirely, but was not.

Results:

"The dose threshold to obtain fast and high mortality was at the slope change, between 40 084 926 and 8 016 985 [spores.cm⁻²](#), where the LT 50 jumped from 3.07 to 3.63 days"

=> Is that really a significant jump ?

Answer: Indeed, it is not a jump, but the interval between these two points corresponds to the transition zone of the exponential decreasing curve, where the slope of the curve goes from close to 1 to close to 0. This is therefore the zone of acceleration of the effect of the dose reduction on the increase in the lifespan of the flies. We changed the wording.

" The LT 50 kept small, 5.64 days, with 2 004 246 [spores.cm⁻²](#) un but reach 22.9 days with ..."

=> what does "un" means? "reach" should either read "reaches" or "reached"

Answer: "un "was deleted. Correction was made.

"1,69 e+3"

=> 1.69 e+3

Answer: Done.

"92.2% ± 0.9 and 99.5% ± 0.2 after 48 h."

=> 92.2 ± 0.9 % and 99.5 ± 0.2 % after 48 h.

Answer: Done.

"Surprisingly, the lowest growth was observed at the intermediate May temperature (20.7-24.3°C)."

=> "Surprisingly" calls for a discussion. Delete it from here and make sure to discuss it in the discussion section.

Answer: Done.

"Survival of flies was negatively affected by spore inoculation"

=> Do you mean method of inoculation or inoculation load?

Answer: *Here we did not vary the amount of spores, we only meant that the flies were inoculated or not. A precision was added.*

"Sex was the only factor having only main effect "

=> Clarify what you mean

Answer: *The sentence was rephrased.*

"but this effect might be interpreted cautiously because it was unbalanced between fertile and sterile population."

=> That sounds like a discussion element

Answer: *The sentence was moved to the discussion section.*

"but significant interaction between adjuvant and month temperature (table 3) revealed"

=> revealed

Answer: *Done.*

Review by Papa Djibril Faye

Evaluation of the different components of the article

Title :

The title is correct but could be reworded for better understanding.

Answer : *The title has been lightened.*

Abstract

The summary reflects the results in the body of the document. However, the last paragraph of the summary should be reworded without emphasizing the central importance of the results.

Answer: *The sentence was moderated.*

Introduction

The introduction highlights the importance of entomopathogens, such as bacteria, as biological control agents in tropical agroecosystems. Their use offers several advantages, including their safety for humans, medium to high specificity and low risk of resistance.

A strain of **Metarhizium anisopliae** has been identified as promising for controlling oriental fruit fly in Africa.

Innovative strategies, such as self-inoculation of wild flies in the field or the release of mass-reared sterile males, are being explored for more sustainable fruit fly control.

The study also examined the effect of insect sex on immunity. The study aimed to assess the virulence of **M. anisopliae** spores on adult **B. dorsalis** flies as a function of inoculation dose, actual spore load, formulation and seasonal air temperature.

Materials and methods

The methodology used is explicit enough to be repeated or improved for other similar studies.

The statistical analyses described seem appropriate for the study in question. The following is an assessment of each method used:

☑ Cox model: This model is commonly used to analyze survival data. It is appropriate for examining how several factors influence the time to a specific event, such as mortality in this case.

☑ Pearson correlation: This method is used to measure the linear correlation between two variables. It is suitable for examining the relationship between tube doses and fly load.

☑ Probit method: This method is commonly used in toxicology to determine the dose of a substance that is lethal to a certain percentage of the population. It is suitable for calculating lethal doses and lethal times.

☑ Generalized Linear Model (GLM): GLMs are an extension of ordinary linear models that allow for non-normal errors. The use of a Poisson distribution for germination and a Gaussian distribution for growth seems appropriate given the nature of the data.

Answer: *We thank you for the positive comments*

Results

The results presented meet the objectives of the study.

Tables and figures

Figures and tables are understandable without reference to the body of the article.

They have an appropriate legend.

Discussion

Studies on the dose-mortality relationship in insects, particularly with regard to the application of fungal spores, often lack precise information on the number of spores actually ingested or carried by individuals. This study examined this relationship to determine a "minimum" and "maximum" inoculation dose to induce mortality. It was found that the range between these two thresholds was very narrow, suggesting that a low number of spores can cause flies to die.

However, this mortality could be better explained by fungal penetration of the weakest parts of the fruit fly cuticle: the head and joints.

Answer: *This is what we intend to say in this sentence "In addition, positioning on the insect body affects penetration speed (Leger 1988b)". We have added a new reference to this sentence and provided further clarification in line with this reference.*

The study's conclusion seems well-founded and relevant to its objectives:

1. Standardization of virulence assessment: The study succeeded in providing elements for standardizing the virulence assessment of entomopathogenic fungal strains against adult fruit flies.
2. Low-dose efficacy of Met69OD: The results on the low-dose efficacy of Met69OD against oriental fruit flies are of interest as they may help optimize the use of this strain in control strategies.
3. Dilution effect of the corn starch adjuvant: The discovery of the dilution effect of the corn starch adjuvant is interesting in view of the longer survival time of the vector. This could help adjust product formulation to maximize efficacy. However, it will be imperative to evaluate this mixture in the context of a study on horizontal transmission.

Answer: *This was added.*

4. Temperature-mediated virulence: Understanding how temperature affects Met69OD virulence is crucial. This can help plan product application according to climatic conditions.

Overall, the conclusion of the study seems well supported by the results presented and offers valuable information for improving control strategies against oriental fruit fly. However, as with all scientific

research, it is important to note that these findings should be validated by further studies and field trials to confirm their efficacy and applicability in different conditions and regions.

Answer: This was added.

References

These three references are missing. 36.↵

Answer: The reference was missing for Leger et al. 1988b and Membang et al., 2021 in the text. This has been corrected. We did not find the error for Leger et al. 1988a.

Leger, R. S., Cooper, R. M., & Charnley, A. K. (1988a). The effect of melanization of *Manduca sexta* cuticle on growth and infection by *Metarhizium anisopliae*. *Journal of Invertebrate Pathology*, **52**(3), 459–470.

Réf. croisée [Google Scholar](#)

37.↵

Leger, R. S., Durrands, P. K., Charnley, A. K., & Cooper, R. M. (1988b). Role of extracellular chymoelastase in the virulence of *Metarhizium anisopliae* for *Manduca sexta*. *Journal of invertebrate pathology*, **52**(2), 285–293.

Réf. croisée [Google Scholar](#)

43.↵

Membang, G., Ambang, Z., Mahot, H. C., Kuate, A. F., Fiaboe, K. K. M., & Hanna, R. (2021). Thermal response and horizontal transmission of cameroonian isolates of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*—Candidates for microbial controls of the banana root borer *Cosmopolites sordidus*. *Fungal Ecology*, **50**, 101042.

[Google Scholar](#)

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