

Dear Editor,

Please find enclosed in the following pages our responses to editorial and reviewer comments to our manuscript JoVE 61798 «**Quantitative methods for measuring volatile and non-volatile antifungal activity of biocontrol products against different stages of fungal growth**». These comments were considered with attention. We thank you for giving us the opportunity to further improve our manuscript which we hope now ready for publication in JoVE.

Point to point responses to Editorial comments

1st point raised :

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

Response : Spelling or grammatical errors had been corrected and the manuscript had undergone a professional proofread prior to the first resubmission. The manuscript has been proofread by a second English professional before the second resubmission.

2nd point raised :

Please revise the title for conciseness.

Response: The recommendation of the Editor is well-taken and the title has been revised for conciseness. We then propose: “Method for Measuring Volatile and Non-volatile Antifungal Activity of Biocontrol Products”.

Changes in the ms: The title has been changed to “Method for Measuring Volatile and Non-volatile Antifungal Activity of Biocontrol Products”.

Point to point responses to Peer-Reviewer #1

Point raised by Rewiewer 1:

The authors have not answered all the queries.

Editor's Note: I have added the prior comments from Reviewer 1 here for convenience. Please ensure that all the comments are addressed or rebutted in the rebuttal letter.

Response to Editor: We considered with attention all the different points raised by Reviewer 1 for the first revision. You will find below the copy of our first point-to-point rebuttal letter as requested by Dr Nguyen.

Copy of Reviewer 1 point-to-point rebuttal letter

1st point raised :

The title can be: The quantitative methods to measure antifungal activities of volatile and non volatile biocontrol products against spore germination (different stages of fungal growth).

Response: We appreciate the suggestion of the reviewer to reformulate the title of the manuscript. As one of the advantage of the method described in the protocol is to address the efficacy of antifungal compounds at defined fungal growth stages, we propose to slightly modify the title proposed by the reviewer as follows : “quantitative methods for measuring volatile and non-volatile antifungal activity of biocontrol products against different stages of fungal growth”

Changes in the ms: The title of the manuscript has been changed to “quantitative methods for measuring volatile and non-volatile antifungal activity of biocontrol products against different stages of fungal growth”

2nd point raised :

Abstract:

Technic change to technique

Specified number of spores are spread

Either placed.... Or in contact

Response: All the reformulations highlighted by the reviewer have been applied. Technic has been replaced by technique L23, L70 and L83. “Either in contact” L33 of the previous manuscript has been replaced by “or in contact” L29 of the revised manuscript.

3rd point raised :

Introduction

It is too lengthy. First para is not necessary. Line 104-131 not necessary. Line 96-102- can be shifted at the end. Avoid repetition

Response: The appreciation of the reviewer is well-taken. We followed the recommendation to shorten and improve the introduction. The paragraph introducing food spoilage and chemical fungicide use (L104-131) has been reduced to present plant-derived products benefits and has been placed at the beginning of the introduction to follow the recommendations of reviewer 3.

The point developed L96-102 of the submitted manuscript have been reformulated and shifted at the end of the introduction (L107-114 of the revised manuscript)

4th point raised

Protocol

Trichoderma- have you deposited the culture in a recognized Culture Collection? If so then give the deposit number.

Response: *Trichoderma* has been isolated from wood has been characterized in macroscopic (comparison of phenotypes on different media) and microscopic (conidia and conidiophore observations) studies. This strain belongs to the genus *Trichoderma* and is likely to be a member of *harzianum* or *viride* species.

Changes in the ms : A note has been added in the ms L124-125 of the revised ms : “*Trichoderma* spp. has been isolated from wood and is used as the model in this study for its rapid growth and ease of spore recovery. This strain is preserved by our laboratory”

Recover conidia- How do authors make sure that there is no contaminating mycelial fragments? The filtration (through sterile muslin cloth) can probably give the uniform suspension

Response: Spores preparations are consistently checked by microscopy upon collection of the samples. In our hands, contamination with mycelial fragments was not observed when using *Trichoderma*. Colonies are velvety and no floccose is present thereby low amounts of pressure is required to get spores detached. This is likely reducing the risk of detaching mycelium. We do nevertheless agree that this point has to be taken into consideration when using fungi displaying recalcitrant conidiophores. We therefore notified in the protocol that an optional filtration step is required in that case.

Changes in the ms: A note has been added in the revised ms L140-143 “This step must be performed carefully to prevent hyphae from being extracted. Spore preparation is then checked under microscope. Eventually, for strains presenting highly aerial and fluffy mycelium, a step of filtration using 40 µM strainer filter can be added to eliminate residual mycelium fragment”

2.1. As I understand the spore suspension (100µl, 3×10^6) is spread over the plate. What is the % viability of spores? The further numbers should reflect viable spores.

Response: Spores are collected from a 4 days culture of *Trichoderma* on PDA and used immediately for experiment for optimal viability. Thus, viability classically approximates 95-98%.

5th point raised

Discussion

It is again too lengthy, which in my opinion is not necessary for JoVE. Delete 425-428. Line 437-457 are presenting observations which are already mentioned under results.

Response: L425-428 and L437-440 have been deleted from the discussion section. To follow Editor recommendations, the discussion has been restructured into 4 paragraphs covering 1) modifications, trouble shooting and critical steps within the protocol, 2) limitations of the techniques, 3) significance in comparison to other methods. The points tackled L441-457 have then been dispatched to follow these recommendations.

Changes in the ms: Discussion text has been restructured and revised

6th point raised

In my opinion, writing is too clumsy and so figures too. The authors can give data only for spore germination inhibition and not for mycelial growth, etc.. which is more complicated. The quantitative results can be explicitly mentioned in Table form and not in figures. The JoVE is for visualization of experiments. The figures depicting protocols need more simplification.

Finally the authors can take help of English speaking person to correct m/s.

Response: Several changes have been realized in the manuscript to improve readability of the introduction and the discussion. Final manuscript has been proofread by a professional English scientific writer. We do agree that previous Figure 1 depicting the protocol lacked readability due to the important level of detail displayed. We thus simplified this figure, which we hope now allows easy visualization and quick understanding of the protocol. Figures 3B and 4 have been merged in the figure 3 of the revised manuscript to illustrate all the capacities of the method in a synthetic format: side-by side benchmarking of multiple products, comparison of volatile and non-volatile antifungal effects, efficacy of the products against specified growth stages. Bar charts have replaced scatter charts to gain visibility as recommended by reviewer 3.

Changes in the ms: Figures have been restructured to facilitate readability and representative results text has been restructured and revised accordingly.

Response to Peer-Reviewer #3

Point raised :

Minor Concerns:

I strongly recommend to indicate the code of Trichoderma strain used in this work to identify it unequivocally, since the sensitivity to the tested antifungal could be strain-depending.

Response: We appreciate the suggestion of the Reviewer to indicate the code of Trichoderma strain originating from our own collection. This code is SBT10-2018.

Changes in the ms: The code of Trichoderma strain SBT10-2018 has been added L120, L124 and L323 of the revised manuscript.