Chapter 21

Preliminary In Vitro Tests to Demonstrate Various Formulations' Effect on the Stability of the Moroccan Trichoderma asperellum-Based Product's Biology

Abdellatif Ouazzani Chahdi
https://orcid.org/0009-0004-9880-8311
Ibn Tofail University, Morocco

Fadoua Berber
Ibn Tofail University, Morocco

Amina Ouazzani Touhami
https://orcid.org/0000-0001-9970-7724
Ibn Tofail University, Morocco

karima Selmaoui
Ibn Tofail University, Morocco

Rachid Benkirane
Ibn Tofail University, Morocco

Allal Douira
Ibn Tofail University, Morocco

ABSTRACT

The selection of the T asperellum-based product required several in vitro tests in order to preserve its efficacy during the formulation process, while maintaining the viability of the conidia and safeguarding their antagonistic properties. The addition of talc to all of the compounds added to the formulation of T asperellum's conidia, Moroccan strain isolated from compost, preserved their viability, stability and antagonistic power during the entire duration under storage conditions. P1 and P2's conidia germination percentages while stored for 38 weeks at room temperature were 0% - 12% and at 4°C were 31% - 46.33% respectively. The antagonistic effect of P1 and P2 against V dahliae by direct contact is estimated by the T asperellum colonization percentages, the mycelial growth of V dahliae's inhibition percentages, the mycelial growth of V dahliae's inhibition percentages and the mycelial inhibition percentages by antibiosis are respectively 0 - 79.58%, 0 - 39.24% and 3.25 - 39.84% at room temperature and 80.22 - 81.49%, 39.83 - 44.99% and 42.27 - 43.09% at 4°C.

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1. INTRODUCTION

Biological control is an important promising avenue to control plant pathogenic diseases and reduce the damage caused by the intensive use of chemical pesticides.

In this context, several researchers have declared that species of the *Trichoderma* genus and in particular *Trichoderma viride* and *Trichoderma harzianum* are the best antagonists against several soil and seed phypathogens (Poddar *et al.,* 2004).

The biological control agent formulated product's shelf life plays an important role in the success of commercialization (Sanjeev et al., 2014). And to maintain the duration of viability of biological control agents, several works were carried out using different adjuvants at different formulations. Among these works, talc, peat, lignite and kaolin preparations of *Trichoderma* have a shelf life of 3-4 months (Sanjeev et al., 2014).

The PDBC (Project Directorate of Biological Control) in Bangalore is working on increasing the shelf life of talc-based *Trichoderma* formulations by using various ingredients (chitin and glycerol) in the production medium and heat shock at the end of the fermentation phase which allows shelf life to be extended up to one year (Sriram et al., 2010, 2011).

The objective of this study is to demonstrate, *in vitro,* the effects of various formulations on the viability and antagonistic power of *Trichoderma asperellum* based product.

2. MATERIALS AND METHODS

2.1 Effect of the Protectant on the Viability and Antagonistic Power of *Trichoderma Asperellum* During Storage

Two organic products based on a Moroccan strain of *T. asperellum*'s conidia registered under the code "Banklt1902509 SMis 1 KU987252 RAB 95369", one of which is formulated using talc (protectant) registered under patent MA 41534B2 (Ouazzani Chahdi et al., 2019). named P2 and the other without the protectant named P1. With the exception of the protectant, the other additives are similar in both products.

2.2 *In vitro* Tests of the Two Biological Products P1 and P2 Against *Verticillium Dahliae* Testing the Viability of Formulated P1 and P2 Conidia

Conidial suspensions from the two products formulated based on *Trichoderma asperellum*: P1 and P2 were prepared and adjusted to a concentration of 10^3 conidia/ml using the Malassez slide.

In boxes containing 20 ml of agar water (15 g of Agar-agar, 1000 ml distilled water), 0.1 ml of each of the two suspensions was spread, with three repetitions for each product. After 24 hours of incubation in the dark at 28°C, the conidia's germination percentage was determined by counting the spores that had germinated in function of the total number of spores.