OPTIMIZATION OF A SOLID SUBSTRATE MIXTURE FOR NEMATOPHAGOUS FUNGI GROWTH AND SPORE PRODUCTION

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Introduction. The solid state fermentation (SSF) is generally defined as the cultivation of microorganisms on a solid support in the absence or near-absence of free water [1]. It is both an ancient and an innovative method, with much less water consumption. It is perfectly suited to the biology of filamentous fungi. Its nature and eco-friendliness makes it a prime candidate to enter a sustainable development concept. This technique can be fruitfully used to produce biopesticides (mainly against plant-parasitic nematodes). The aim of this work was to demonstrate that the use of a mixture (sugar cane bagasse + wheat bran + potato + chitin from shrimps) offers good growth conditions for *Paecilomyces lilacinus,Trichoderma harzianum* spore production in SSF with or without hydric stress.

Methods. *P. lilacinus* and *T. harzianum* were maintained and cultivated in Potato Dextrose Agar (PDA) for estimation of apical growth and obtention of spore suspension. **Substrates:** 0.8-2.2 mm mixture of sugar cane bagasse (50 g) + wheat bran (30g)+ mashed potato (18g)+ chitin from shrimp (2 g)+ Water with or without mineral solution (100 mL). The culture medium was sterilized at 15 lb, and 121ºC for 60 min. The initial water content was 65 or 75% obtained with an inoculation rate of 2×10^7 sp.g^{-1}; the incubation at 25°C was maintained for 7 or 14 days. The sporulation yield was defined as the number of spore produced per gram of solid mixture dry weight using a Malassez hematimeter cell. Table 1 shows the estimated variables and analysis.

Table 1 Estimated variables and analysis.

N°	X = factors	Y= results
1	Initial humidity	Sporulation yield
2	Temperature	Viability yield
3	Chitine substrate	Virulence yield
4	Mineral salts	Humidity
5	Hydric stress	CO_2 rate
6	Incubation time 7 or 14 days	Cellulases rate
7	Settlement	Chitinases rate
8	Aeration flow	Long-term conservation
9	Irradiance	

Analysis
Water content; spore count; spore viability and virulence, as well as enzyme analysis (cellulases and chitinase), were performed from fresh and dried fermented products.

Results

Figure 1 shows the macroscopic fungal observations:

Fig.1a. *T. Fharzianum* colony on PDA

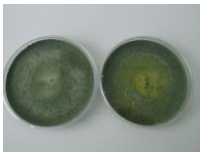
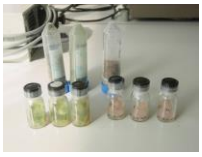


Fig.1b. *T. harzianum* and *P. lilacinus* strains



Growth on PDA susbtrate, 25°C during 5 days :

Apical growth	*T. harzianum*	*P. lilacinus*
mm/D	20,67 mm/D	2,53 mm/D

Sporulation yields, at 25°C (sp.g^{-1} of carbon source):

Medium	*T. harzianum*	*P. lilacinus*
PDA 7D	1,04E+10	2,26E+10
PDA 14D	1,02E+10	2,21E+10
Mixture 66% water 7D	3,07E+09	2,16E+10
Mixture 66% water 14D	7,16E+09	3,55E+10
Mixture 75% water 7D	1,03E+10	-
Mixture 75% water 14D	1,42E+10	-

Conclusion

This study showed that the optimum conidiospore production was obtained after 7 days of SSF at 75% for *T. harzianum* and 66% for *P. lilacinus*. The mixture of solid agricultural byproducts is a good substrate for the production by SSF of high amounts of spores of both strains that can be used as a biological control agent.

References:
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