



Diversity and Biocontrol Efficacy of Entomopathogenic Fungi from Portuguese Chestnut Groves (Soutos) for Integrated and Sustainable Pest Management



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INTRODUCTION

Sweet chestnut (*Castanea sativa* Mill.) is economically and ecologically important in Portugal but is severely affected by key pests, particularly chestnut weevil and tortricid moths ("bichados") (Fig. 1) [1,2]. Due to environmental and health concerns associated with chemical pesticides, sustainable alternatives aligned with EU policies are urgently needed. Entomopathogenic fungi (EPFs) are promising biocontrol agents, yet their use in chestnut orchards (soutos) remains underexplored.

Objectives: (1) Isolate, identify, and assess the diversity of EPFs from chestnut orchard soils in experimental plots in Trancoso; and (2) Test their field efficacy compared with the commercial insecticides *Affirm Opti*® and *Spintor 480 SC*® for controlling chestnut pests.



Fig. 1: Chestnut pests (bichados): A – adult; B – larva

1. SAMPLING AND FIELD TRIAL LOCATION

Experimental plots in Trancoso were used for both soil sampling and field trials (Fig. 5).

2. INSECT-BAIT METHOD

EPFs were isolated using *Tenebrio molitor* larvae as insect bait and incubated at 28 °C and 80% humidity (Fig. 2).

4. IDENTIFICATION OF EPFs (Fig. 4).

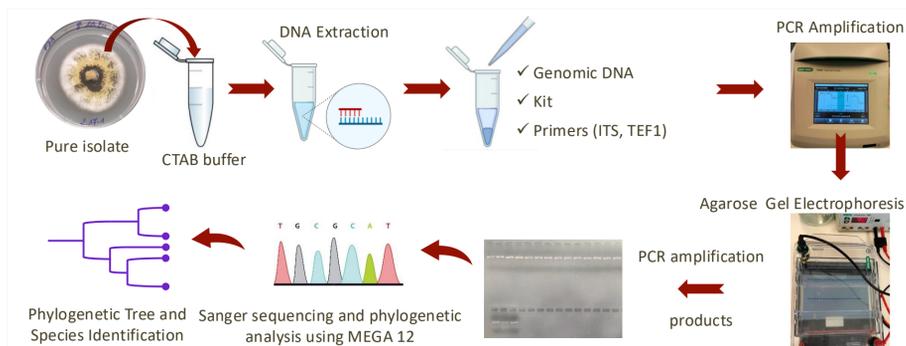


Fig. 4: Process of molecular identification of isolated EPFs.

MATERIALS AND METHODS



Fig. 2: Insect-bait method. A: Petri dish; B: Growth chamber.

3. ISOLATION OF EPFs

Infected larvae were surface-sterilized and plated onto RBCA and PDA media (Fig. 3).



Fig. 3: EPFs isolation and purification process.

5. FIELD TRIALS: APPLIED TREATMENTS

Five EPF isolates and two commercial insecticides were tested (Fig. 5; Table 1).

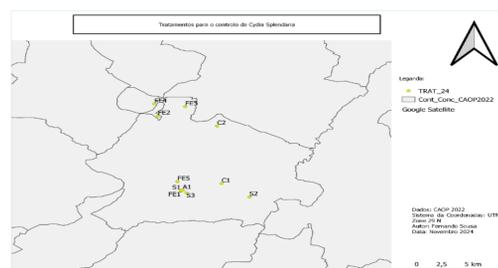


Fig. 5: Location of the applied treatments.

Table 1: Additional information on the sampling points.

Treatment	Product	Active Substance	Local	Parish (Administrative Unit)	Altitude (m)
A1	<i>Affirm Opti</i>	Emamectin	Trancoso	U. F. Trancoso and Souto Maior	845
S1	<i>Spintor</i>	Spinosad	Trancoso	U. F. Trancoso and Souto Maior	845
S2	<i>Spintor</i>	Spinosad	Feital	U. F. Vila Franca das Naves e Feital	714
S3	<i>Spintor</i>	Spinosad	Frechão	U. F. Trancoso and Souto Maior	817
FE1	<i>M. robertsii I</i>		Trancoso	U. F. Trancoso and Souto Maior	846
FE2	<i>M. robertsii I</i>		Sebadelhe	U. F. Torre do Terrenho, Sebadelhe da Serra and Terrenho	925
FE3	<i>M. robertsii II</i>		Torre Terrenho	U. F. Torre do Terrenho, Sebadelhe da Serra and Terrenho	822
FE4	<i>M. robertsii II</i>		Guilheiro	Guilheiro	828
FE5	<i>B. bassiana</i>		Trancoso	U. F. Trancoso and Souto Maior	845
C1	-	-	Tamanhos	Tamanhos	804
C2	-	-	A-Do-Cavalo	Morreira de Rei	793

i. ISOLATION AND DIVERSITY OF EPF IN CHESTNUT-ORCHARD SOILS

Six EPF species were isolated and identified, representing four genera (Fig. 6). *Clonostachys* showed the highest diversity, with three species identified.

M. robertsii was the dominant species in most samples, followed by *B. bassiana* as the second most prevalent species (Figs. 7 and 8).

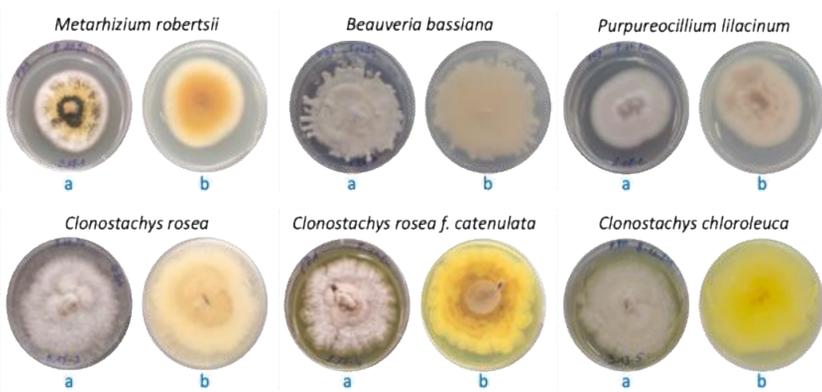


Fig. 6: Morphology of identified EPF colonies on PDA (a) front view and (b) reverse view.

ii. EFFICACY OF THE TREATMENTS FOR CONTROLLING CHESTNUT PESTS

Figures 11–13 show the efficacy of EPF isolates and commercial insecticides against chestnut pests. Overall, EPF treatments demonstrated high efficacy, with FE5 (*B. bassiana*) showing the best performance (100%) across all pest species, followed by FE4 (*M. robertsii II*). The chemical treatment (A1) exhibited the lowest efficacy.

RESULTS

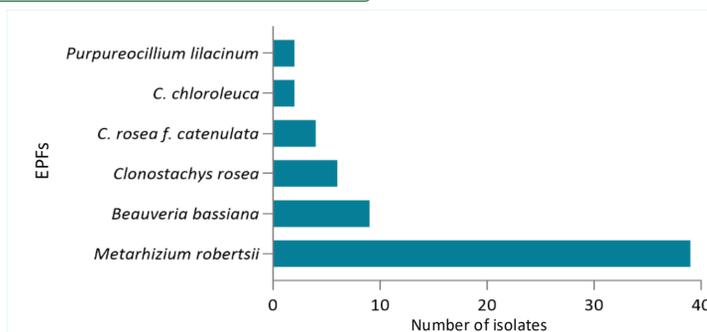


Fig. 7: Abundance of EPF species isolated from chestnut soils.

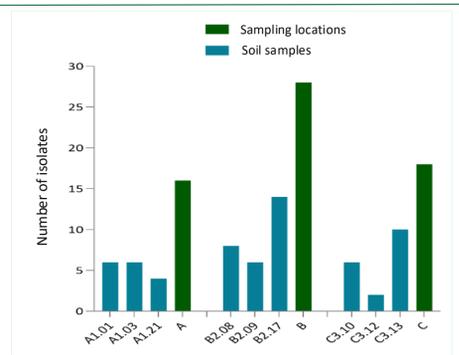


Fig. 10: Abundance of EPF isolates across sampling sites and soil samples

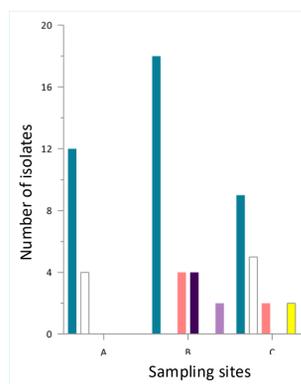


Fig. 8: Distribution of EPF species across the three sampling sites.

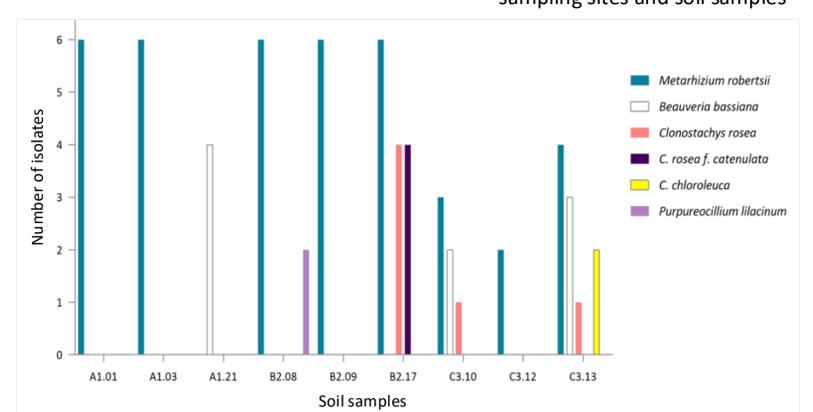


Fig. 9: Distribution of EPF species across soil samples from the three sampling sites.

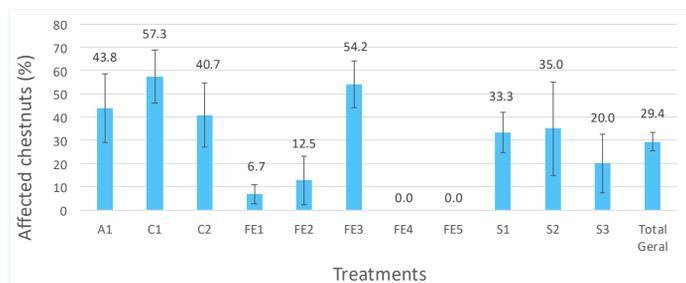


Fig. 11: Mean ± SD percentage of chestnuts attacked by *P. fasciana* and *C. fagiglandana*

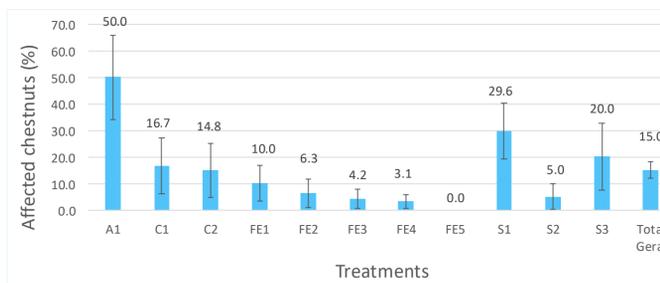


Fig. 12: Mean ± SD percentage of chestnuts attacked by *C. splendana*.

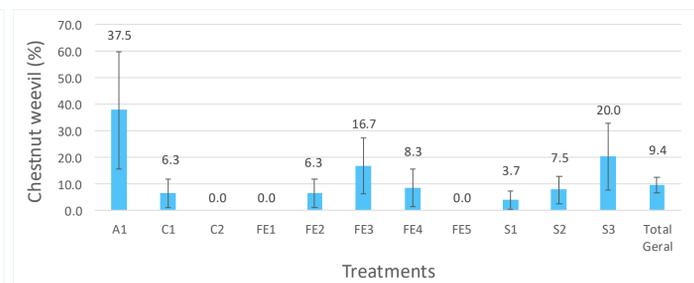


Fig. 13: Mean ± SD percentage of chestnuts attacked by *C. elephas*

CONCLUSIONS

The results indicate that chestnut soils in Trancoso harbor a high diversity and abundance of EPFs, mainly *M. robertsii* and *B. bassiana*. These two native EPFs showed high efficacy in controlling chestnut pests, outperforming the chemical treatment. *B. bassiana* achieved 100% efficacy against all tested pests, followed by the *M. robertsii* isolates. Thus, *B. bassiana* emerges as a promising alternative for sustainable chestnut pest management. Further studies will be conducted to validate these findings and to develop commercial products based on these native EPFs.

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