USE OF CORK RESIDUES TO CONTROL TURFGRASS DISEASES

Coelho, L.[1,2]; Dionísio, L. [1,2]; Reis, M. [1,2] and Guerrero, C. [1,2,3]
[1] Universidade do Algarve, Faculdade de Ciências e Tecnologia
[2] MedtiBio, Centre for Mediterranean Bioresources and Food
Faro, Portugal
[3] E-mail: cguerre@ualg.pt

Introduction

The worldwide increase in agricultural and industrial production has created environmental problems. Economic and environmental benefits can be gathered solving a problem of the agroindustry by applying their sub products to soil. The compromise to decrease the use of pesticides and fertilizers, which may be hazardous, has provided opportunities for the development of new sustainable crop management practices. From several strategies to enhance the use of organic matter in agriculture, one has been the use of composts of different mixtures of raw materials, from different agroindustry processes or the use of these raw material (agroindustry residues) directly without any treatment. The incorporation of these products to the soil and its application to the crops proved to be an interesting pathway to apply effective beneficial microorganisms for the crops and for the ecosystems globally. This strategy showed to achieve reasonable crops yields and suppressive effects on phytopathogenic microorganisms. Several microorganisms have been associated to cork throughout tree life and in the end products[1], such as *Trichoderma pseudoconingii*, *T. viride*, *Endothiella gyrosa, Mucor hiemalis*, *Rhysopus* sp., *Penicillium* sp., *Cytospora* sp., *Dichomera* sp., *Acremonium* sp., *Glyocladium* sp., *Botrytis silvatica*, and *Pestalotia* sp. Considering the potential of these microorganisms, a study was carried out at the University of Algarve to identify the presence of beneficial microorganisms in cork residues and to evaluate, *in vitro,* their antagonistic effect against several fungi turfgrass diseases.

Material and Methods

Physical, chemical and microbiological characteristics of residues from cork transformation industry (NOVACORTIÇA, SA, Portugal), were performed according to methodologies described by[2]. For this study, an extract from cork residues was prepared in a Ringer solution at a dilution of 10^{-1}, followed by decimal dilutions. Potato dextrose agar medium (PDA) was used to isolate and quantify fungi populations in the extract; Plate Count Agar (PCA) for heterotrophic bacteria and PCA medium at half the manufacturer's recommended concentration (1/2 PCA) for actinomycetes. Culture media were surface inoculated with 100 µL of a serial of dilutions of the cork extract and incubated at 25 °C ± 1 °C for 24 hours, in the dark[2]. The isolation and identification of the fungi was done by microscopy and by molecular biology techniques. DNA was extracted from mycelium grown in PDA medium. The obtained DNA samples were subjected to Polymerase Chain Reaction (PCR) using the primers ITS1 and ITS4[3], and the product obtained was sequenced. The antagonistic capacity of the isolated *Trichoderma harzianum* was evaluated according to the method of direct confrontation, described by[4] and its inhibition rate was tested against turfgrass pathogenic fungi: *Rhizoctonia solani*, *Clarireedia* spp., *Sclerotium rolfsii*, *Alternaria alternata*, *Fusarium oxysporum* and *Colletotrichum* spp. The confront direct tests were carried out in Petri dishes with PDA, using two 6.5 mm diameter discs: one with the pathogen mycelium and the other with the antagonist mycelium, 3 cm apart. To determine the percentage of inhibition (PI), each tested fungi was grown alone in PDA where a 6.5 mm discs with its mycelium was placed in the center of the culture medium. The ratio of the growth zones of each fungus were measured daily. All the assays were run in triplicate.

---

[1] Santos, M.N.; Bragança, M.H. & Casimiro, P.P. 2005. Microrganismos Associados à Cortiça em Diferentes Fases da sua Fileira. *Silva Lusitana*, **13**(1): 75 – 93.

[2] Coelho, L., Reis, M. & Dionísio, L. 2013. Variation in microbial populations during composting of agro-industrial wastes. Applied Microbiology and Biotechnology 97: 4179-4186.

[3] White, T.J.; Bruns, T.; Lee, S. & Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Sninsky, J.J. & White, T.J. (Eds.) *-PCR protocols: A guide to methods and applications*. New York, New York, Academic Press, p. 315-322.

[4] Magan, N. & Lacey, J. (1984) – Effect of water activity, temperature and substrate on interaction between field and storage fungi. *Transactions of the British Mycological Society*, vol. 82, n. 1, p. 83-93. https://doi.org/10.1016/S0007-1536(84)80214-4

Results and Discussion

The cork residue had a high organic matter content, as recommended for agricultural use[5]. The pH is lightly acid and the electrical conductivity is compatible with the agricultural use[6]. According to[7], cork residues presents suitable properties (Table 1) to be used as plant growing media.

Table 1: Physical and chemical properties of the cork residue.

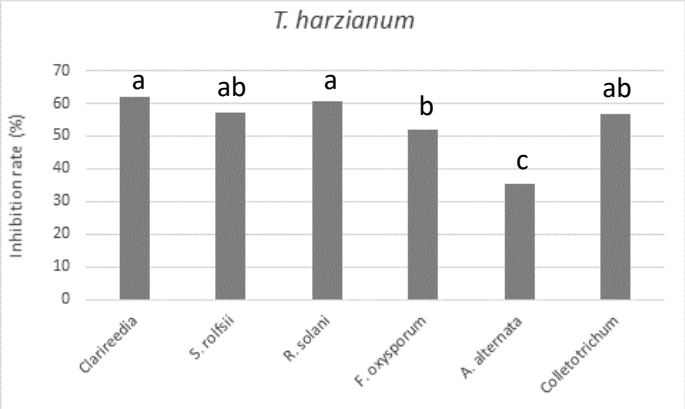
pH	CE (dS cm^{-1})	DM (%)	OM (%)	RD	BD	AC (%)	EAW (%)	RW (%)	DAW (%)
6.02	0.2	54.1	94.7	1.48	0.229	19.6	24.2	5.0	35.7

CE, electrical conductivity; DM, dry matter; OM, organic matter; RD, real density; BD, bulk density; AC, air capacity; EAW, easily available water; RW, reserve water; DAW, difficult available water.

Cork residues showed high microorganisms' populations (Table 2), namely fungi, such as: *Penicillium* spp., *Aspergillus* spp., *Mucor* spp. and *Trichoderma harzianum*.

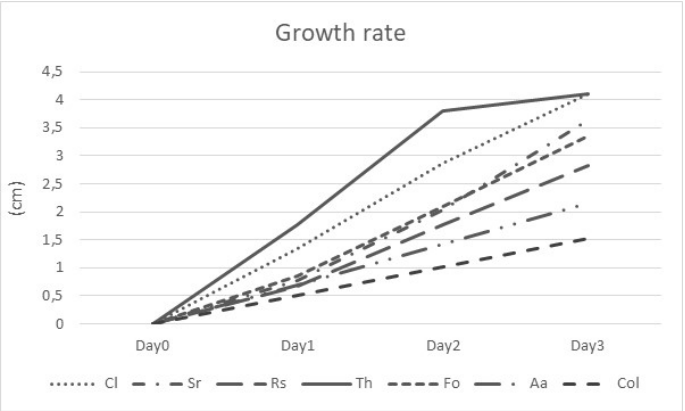
Fungi	Bacteria	Actinomycetes
1.50×10^7	1.43×10^6	1.57×10^6

Table 2: Microorganisms' populations (CFU.g $cork^{-1}$) quantified in the cork residues.



Trichoderma harzianum isolated from the cork residues was tested by direct confrontation technique. For the diseases studied, the inhibition rate was higher than 50 %, except for *A. alternata* (35.4 %). The inhibition rate was higher for *Clarireedia* spp. and *R. solani*, with values above 60 % (Figure 1).

Figure 1: Inhibition rate by direct confrontation between *Trichoderma harzianum* and the tested phytopathogenic fungi. Bars with the same letter have no statistically significant differences for $p<0,05$ (Duncan test).



T. harzianum showed the fastest growth rate until day 2. On day 3, both *T. harzianum* and *Clarireedia* spp. mycelia occupied all the surface area of the culture media (Figure 2). However, despite *Clarireedia*'s high growth rate, *T. harzianum* was able to inhibit its growth.

Figure 2: Growth rate of *Trichoderma harzianum* and the tested phytopathogenic fungi. Cl, *Clarireedia* spp.; Sr, *Sclerotium rolfsii*; Rs, *Rhizoctonia solani*; Ta, *Trichoderma harzianum*; Fo, *Fusarium oxysporium*; Aa, *Alternaria alternata*; Col, *Colletotrichum* spp.

Since the fungi isolated from the cork residues had a positive effect on turf diseases control *in vitro*, further work is being planned to study the effect of the cork extract on turfgrass diseases under field conditions.

[5] Ferreira, J., Conceição, J., Strecht, A., Ribeiro, J., Soeiro, A. & Cotrim, G. 2002. Manual de agricultura biológica – Fertilização e proteção das plantas para uma agricultura sustentável. Agrobio. 3ª Edição. Lisboa. pp. 435.

[6] Brinton, W. 2000 - *Compost quality standards & guidelines - Final Report.* Woods End Research Laboratory, Inc. Available at http://compost.css.cornell.edu/Brinton.pdf. Access in January, 2019.

[7] Abad, M., Noguera, P. & Carrión, C. 2004. Los substratos en los cultivos sin suelo. In: Abad et al., M. U. 2004. Tratado de cultivo sin suelo. Ediciones Mundi-Prensa. 3ª Edição. pp. 113-158.