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ANTAGONISTIC POTENTIAL OF *TRICHODERMA HARZIANUM* AGAINST POSTHARVEST FUNGAL PATHOGENS

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INTRODUCTION

Postharvest decay is a serious problem in the storage of many fresh fruits and vegetables. Introduction of non-chemical control methods to reduce postharvest decay is becoming increasingly important. Consumers are demanding less chemical residue on produce, and many fungi are developing resistance to commonly used fungicides. Moreover, the use of chemical fungicides is becoming more restricted due to environmental and health concerns (Janisiewicz and Korsten, 2002). It is therefore necessary to develop alternatives to synthetic fungicide to reduce environmental risks and raise consumer confidence. Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods. *Trichoderma harizanum*, *T. viridae*, *T. virens*, *T. hamatum, T. roseum* and *T. koningii* are the most common fungal biological control agents (BCAs) that have been comprehensively researched and deployed throughout the world. Major mechanisms involved in the biocontrol activity of *Trichoderma* spp. were competition for space and nutrients, production of diffusible and/or volatile antibiotics, and hydrolytic enzymes like chitinase and β-1,3-glucanase (Howell, 2003).
The objective of the present study was to evaluate the inhibitory role of *T. harzianum* in the biological control of some postharvest fungal pathogens.

MATERIAL AND METHODS

Pathogens and antagonist
The monoconidial isolates of postharvest fungal pathogens were isolated from various decayed fruits (Table 1.). Antagonistic microorganism *T. harzianum* (DSM 63059), employed for *in vitro* antimicrobial assay was obtained from German Collection of Microorganisms and Cell Cultures (DSMZ).

Antagonistic activity *in vitro*
The assay for antagonism was performed on PDA in Petri plates by dual culture method. The experiment was repeated twice with three replications of each treatment. Percent growth inhibition (PGI) was calculated using the formula: PGI (%) = R-R1/R x 100, where R represents the distance (measured in mm) from the point of inoculation to the colony margin in control plates, and R1 the distance of fungal growth from the point of inoculation to the colony margin in treated plates in the direction of the antagonist (Korsten and De Jager, 1995). *T. harzianum* was tested for both antibiosis and mycoparasitic activities against isolates of postharvest pathogens. Hyphal interaction and morphology were examined under a light microscope.

Table 1. Postharvest fungal pathogens used to test the antagonistic activity of *T. harzianum.*

Isolate	Species	Host
AAJ- 2	*Alternaria alternata*	apple
ASL-2	*Aspergillus flavus*	lemon
BCJ-1	*Botrytis cinerea*	strawberry
CAJ- 20	*Colletotrichum acutatum*	apple
CGP- 9	*C. gloeosporioides*	sour cherry
MUP- 1	*Mucor* sp.	pear
PEL- 4	*Penicillium expansum*	lemon

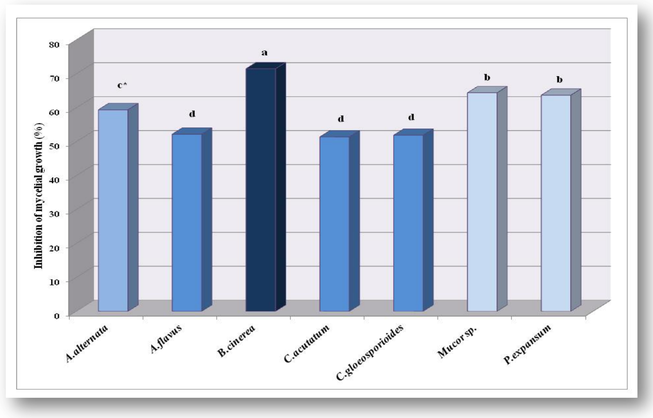

Figure 2. Percent growth inhibition of postharvest fungal pathogens by *T. harzianum*.The values with the same letter are not statistically different by Duncan's multiple range test ($p < 0.05$).

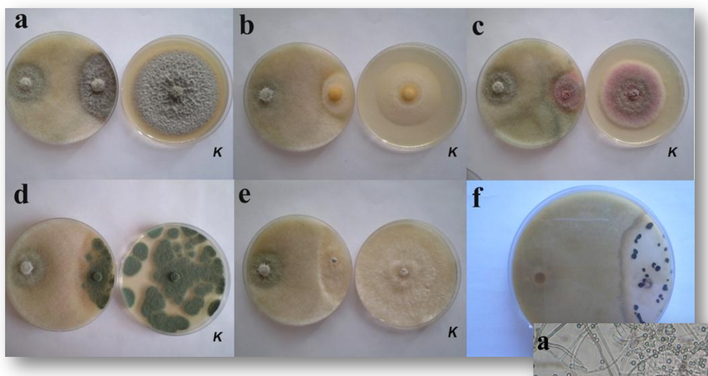

Figure 1. Antagonistic activity of *T. harzianum* against: (a) *A. alternata*; (b) *A. flavus*; (c) *C. acutatum*; (d) *P. expansum*; (e) *B. cinerea*; and (f) mycelial degradation of *B. cinerea* in dual culture assay with *T. harzianum* (back side); K – control plate without antagonist.

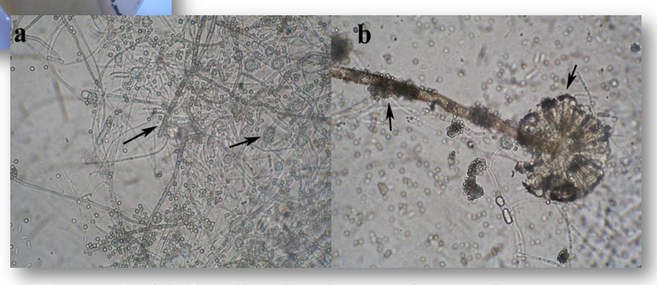

Figure 3. (a) Swollen hyphae and vacuolar appearance of the mycelium of *A. alternata*; (b) degradation of the fungal cell wall of *A. flavus* in dual culture assay with *T. harzianum* (light microscope, 600x).

RESULTS

Results from dual culture assay showed that *T. harzianum* inhibited the mycelial growth of all tested pathogens with a high PGI value (51% - 72%), (Figure 1 and 2). Microscopic examination of dual culture assay showed alternation of the mycelium of pathogen where it was in contact with antagonist. Mycoparasitism of *T. harzianum* was observed as coiling, penetration, degradation of the fungal cell wall, and direct contact and parallel growth alongside host hyphae of *A. alternata*, *A. flavus*, *B. cinerea*, *C. acutatum*, *C. gloeosporioides* and *Mucor* sp. (Figure 3b). Also, *T. harzianum* induced abnormal stunted, highly branched hyphal tips, swollen hyphae and the vacuolar appearance of the mycelium of all tested fungal pathogens.

CONCLUSION

The expression of antagonistic activity by a microorganism towards a pathogen in culture medium cannot generally be taken as evidence of control *in situ*. However, our results show that *T. harzianum* (DSM 63059) have a wide spectrum of antimicrobial activity, and might be used as BCA against various postharvest fungal pathogens.

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