Antagonistic activity of *Penicillium oxalicum* Corrie and Thom, *Penicillium decumbens* Thom and *Trichoderma harzianum* Rifai isolates against fungi, bacteria and insects *in vitro*

Mª Pilar Santamarina[1], Josefa Roselló[1], Reyes Llacer[1] & Vicente Sanchis[2]

[1]Departamento de Biología Vegetal, E.U.I.T.A. Valencia, Spain; [2]Food Technology Dept., University of Lleida, CeRTA, Lleida, Spain

Summary

The antibiotic activity of 70 isolates belonging to the genera *Aspergillus, Penicillium, Fusarium, Alternaria* and *Trichoderma* was tested as preliminary screening. The highest activity was obtained with three *Penicillium oxalicum* isolates, one *Penicillium decumbens* isolate and the *Trichoderma harzianum* isolate. After that, we chose these five isolates in order to carry out other studies with bacteria, fungi and insects. Extracts from these isolates were obtained. The extracts were tested for antibiotic activity with positive results, which implies that metabolite production is involved in this antagonistic effect. The highest activity was shown by *T. harzianum* and *P. oxalicum* extracts, but there was high variability among *P. oxalicum* isolates.

Key words

Bactericidal and fungicidal activity, Secondary metabolites, *Penicillium oxalicum, Penicillium decumbens*, *Trichoderma harzianum*

Actividad antagonista de *Penicillium oxalicum* Corrie and Thom, *Penicillium decumbens* Thom y *Trichoderma harzianum* Rifai frente a hongos, bacterias e insectos *in vitro*

Resumen

En el presente trabajo se realizó un estudio preliminar de la actividad antibiótica de 70 cepas fúngicas pertenecientes a los géneros *Aspergillus, Penicillium, Fusarium, Alternaria* y *Trichoderma.* Los mejores resultados se obtuvieron con tres cepas de *Penicillium oxalicum*, una de *Penicillium decumbens* y la cepa de *Trichoderma harzianum*, por lo que estas cinco cepas se seleccionaron para llevar a cabo otros estudios frente a bacterias, hongos e insectos. Con los extractos obtenidos de cada una de las cepas seleccionadas, se realizaron ensayos para la detección de la actividad bactericida, fungicida e insecticida de los mismos, obteniéndose resultados positivos, lo que implica que la producción de metabolitos activos está involucrada en el efecto antagonista de estos agentes. Los extractos de *T. harzianum* y *P. oxalicum* mostraron los mejores resultados; sin embargo, se observó una gran variabilidad entre las distintas cepas ensayadas de *P. oxalicum.*

Palabras clave

Actividad bactericida y fungicida, Metabolitos secundarios, *Penicillium oxalicum*, *Penicillium decumbens*, *Trichoderma harzianum*

Dirección para correspondencia:
Dr. Mª Pilar Santamarina
Departamento de Biología Vegetal. E. U. I. T. A.
Av. Blasco Ibáñez 21
46010 Valencia, Spain
Tel.: +34 96 387 7414
Fax: +34 96 387 7149
E-mail: mpsantam@bvg.upv.es

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Pesticides play an important role in the stabilization and increase of agricultural yield, but are accused of being a possible source of atmospheric pollution, with residual toxicity to mammals and wildlife.

Microbial products with antimicrobial activity are now being applied in every sphere of pesticide use. Thus, some antifungal, antibacterial, insecticidal and herbicidal products used in crop protection have been obtained from microorganisms [1].

Some species of fungi can secrete substances or metabolites that have very specialized activity, being lethal to a particular group of life forms. Since 1963, fungi have received great attention as biocontrol organisms against pests [2-5].

Extensive work has been carried out in order to identify fungi with potential capabilities as pesticides, thus many screenings for antagonistic activities of fungi can be found in the literature [5-11].

Several new disease biocontrol agents have become commercially available in the recent years, including *Gliocladium virens, Streptomyces griseoviridis, Trichoderma harzianum* and vesicular-arbuscular mycorrhizal fungi. Each has a different pathogen/host target but all are registered for use in protected crops rather than in the field, emphasizing the significance of stable environmental conditions for achieving reproducible biological disease control [12]. Recent advances on soil-borne disease control include: a) development of biocontrol formulation systems for delivery, b) genetic manipulation of biocontrol agents to increase their efficacy; and c) the use of biocontrol in integrated pest management [13].

Research in agriculture has been directed to fungi as biocontrol agents against both other fungi and insects. Most of fungal antagonists, however, have been used because of their antifungal properties [8,14,15].

The purpose of this paper was to study the activity of different *Penicillium, Aspergillus, Fusarium, Alternaria* and *Trichoderma* isolates from cereals and their metabolic broths against both bacteria and fungi, in order to look for some antibacterial and antifungal and insecticidal products.

MATERIALS AND METHODS

Microorganisms. The 70 isolates tested in this study were isolated from cereal samples obtained from the retail market in Valencia (Spain). The distribution of the isolates was: *Penicillium* (32), *Aspergillus* (30), *Fusarium* (7) and *Trichoderma* (1). The reference isolates used in the different tests were obtained from Spanish collections: CECT (Colección Española de Cultivos Tipo), CR (Reus Medicine Faculty Collection) and MTAL (University of Lleida, Food Technology Dept. Collection). All the isolates were maintained in potato dextrose agar (PDA).

Preliminary assay. Wickerham's test was performed on solid media following the classical procedure and the lack of growth in a 1-2 cm width area around the fungal colonies was recorded as the result of an inhibitory effect. The bacterial strains used belonged to the CECT and they were: *Agrobacterium tumefaciens* (Smith and Townsend) Conn (CECT 472), *Bacillus subtilis* (Ehrenberg) Cohn (CECT 356), *Escherichia coli* (Migula) Castellani and Chalmers (CECT 434 and CECT 943), *Pseudomonas solanacearum* (Smith) Smith (CECT 125), *Serratia marcescens* Bizio (CECT 977), *Staphylococcus aureus* Rosenbach (CECT 239), *Streptococcus pyogenes* Rosenbach (CECT 985), *Streptomyces albus* (Rossi Doria) Waksman and Henrici (CECT 3077), and *Xanthomonas campestris* (Pammel) Dowson (CECT 97).

Determination of the bactericidal, fungicidal and insecticidal capacity

Fungal extracts. According to Wickerham's test results a number of isolates (five) were selected for the following steps of the study.

From a seven-day culture of the fungal isolate grown on PDA, a suspension (10^8 spores/ml) was obtained; 1 ml of the suspension was inoculated into Erlenmeyer flasks (500 cc) containing either 100 ml of potato dextrose broth (PDB) or Wickerham broth (WB). Number of flasks depended on the amount of extract required.

Cultures were incubated at 28 °C in the dark for 14 days. After that, the mycelium was collected by filtration through Whatman 4 paper. Culture supernatants were extracted by washing them three times with 50 ml of dichloromethane. The extracts were dehydrated by passing them through a layer of anhydrous Na2SO4, concentrated in a rotavapour and evaporated to dryness under a stream of nitrogen. Later, they were weighted and redissolved in either cyclohexane, acetone or a mixture of both depending on the solubility of each one. Extracts from cultures grown both in Wickerham and potato dextrose broth were assayed in the following sections.

Bactericidal capacity. The method consists of determining the inhibition of the bacterial growth, on impregnating the fungal extracts on discs, using in the test, solid media (Mueller-Hinton agar). Bacteria were grown in a broth medium and 0.5 ml of this broth (10^8 UFC/ml) inoculated by inclusion, then impregnated paper disks (5 mm diameter) were placed on the agar surface. For each tested bacteria, two different assays were carried out, one containing 50µg of extracts in each disk, and another containing 100µg of extracts in each disk. The following bacteria were used in this test: *A. tumefaciens* (Smith and Townsend) Conn (CECT 472), *E. coli* (Migula) Castellani and Chalmers (CECT 943), *P. solanacearum* (Smith) Smith (CECT 125), *X. campestris* (Pammel) Dowson (CECT 97), *S. marcescens* Bizio (CECT 977). They were incubated at 37 °C and the reading was taken 24 h later, by measuring the inhibition halo produced.

Fungicidal capacity. The method consists of determining the inhibition of fungal growth, on impregnating the fungal extracts on disks, using in the test, solid media (PDA). One ml of a spore suspension (10^8 spores/ml) of each fungus were inoculated by inclusion, then impregnated paper disks (5 mm diameter) were placed on the agar surface. Two different assays were carried out in which the quantities of the extract on the disks were 50 and 100 µg. The following fungi were used: *Aspergillus candidus* (CR -Reus, Medicine Faculty Collection, Spain-100), *Aspergillus versicolor* (Vuillemin) Tiraboschi (CECT 2890), *Penicillium griseofulvum* Dierckx (MTAL -University of Lleida, Food Technology Dept. Collection, Spain- 3.88), *Curvularia trifolii* Parmelee and Luttrel (CECT 2863) and *Botrytis cinerea* (Persoon) Fries (CECT 2100). They were incubated at 28 °C and the reading was taken five days later, by measuring the inhibition halo produced.

Insecticidal capacity. Maturation induction activity against Oncopeltus fasciatus. *Oncopeltus fasciatus* used in this test, came from a colony kept in the laboratory at 29 ºC, with a relative humidity between 65-70%.

For this test, extracts were evaporated to dryness and dissolved in cyclohexane, acetone or a mixture of both depending on solubility until reaching a concentration of 250 µl/ml. Then 130 µl of this dilution were placed in a Petri dish plus two additional ml of disolvent, giving

a residual deposit of 500 µg/cm². Once the dish was dry, 15 individuals in the nymph state were placed in along with appropriate feed. Incubation was carried out at relative humidity of 60-70%, at 29 ºC and with a constant photoperiod for 14 days, the toxicity of the extracts was studied, by determining the percentage of dead individuals after three, seven, and 14 days.

An additional experiment was carried out by transferring survivors after three days to containers under suitable conditions in order to observe the ones that reach adult stage as well as precocious adults.

RESULTS AND DISCUSSION

Preliminary test. Out of 70 isolates assayed, 37 (52.11%) were able to exert an inhibitory effect against at least one of the bacteria tested. The percentages depended on the genus they belonged to: *Aspergillus* (17 out of 30), *Penicillium* (24 out of 32), *Fusarium* (one out of three), and *Trichoderma* (one out of one). Most of the isolates with positive results belonged to *Penicillium* genus, which is well known because of the ability of its species to produce antibiotics, some of them at an industrial scale. Moreover, *Penicillium* species inhibited a wider range of bacteria. *P. griseofulvum* has been reported to be an important inhibitor of bacterial growth; this ability has been associated with its griseofulvin and patulin production [16].

Aspergillus isolates showed an inhibitory activity mainly against *S. aureus*, one *E. coli* isolate and *S. albus*, while *Penicillium* isolates were effective mainly against *B. subtilis*, *S. albus*, *S. aureus* and *S. pyogenes*. Previously, *Penicillium* isolates from dry-cured ham had shown wide antibiotic effects when tested against both bacteria and yeast, similarly they observed a high sensitivity in *E. coli, B. subtilis* and *S. aureus* isolates, while *S. marcescens* displayed a weak sensitivity. By contrast, little sensitivity was shown by *S. marcescens, S. aureus* and *B. subtilis* against *Aspergillus* species, concluding as in the present study that *Penicillium* spp. beard wider antibiotic capabilities[17]. Among *Penicillium* and *Aspergillus* species isolated from vineyard soils, *P. oxalicum* was one of the species with a broader spectrum of activity against *S. aureus, B. subtilis, B. cereus, P. mirabilis* and *Candida albicans* [9].

The only *T. harzianum* isolate tested showed a wide spectrum of inhibition. Fungi of this genus have been successfully applied for seed treatments [18]. *T. harzianum* has also been applied in soil, and *T. koningii* on cowpea leaves, as a biocontrol agent against *Rhizoctonia solani* on cotton in a greenhouse environment [19,20], and against wood degrading fungi [21]. Both *T. viride* and *T. harzianum* are recognized biopesticides mainly against *Rhizoctonia, Sclerotinia* and *Botrytis* [13]. In *in vitro* studies, *Rhizoctonia solani, Pythium ultimum* and *Chalara alegans* were strongly inhibited by *Trichoderma viride, T. harzianum, T. pseudokoningii* and *T. koningii*, both when the pathogen and antagonist were grown in pairs in the same agarized medium and when they were grown on separate media in a confined environment. These results indicate that the biocontrol efficacy of *Trichoderma* seems to perform not only at medium, but also at atmosphere level [15]. Effect of volatiles produced by *T. hamatum* on growth of phytopathogen fungi from soil has been shown [22].

The best results were obtained by three *Penicillium oxalicum* isolates, one isolate of *P. decumbens*, and one *T. harzianum* isolate, which were used in the following sections. All these species are distributed world-wide. *P. oxalicum* is especially common in soil and upon various organic materials undergoing slow deterioration or decay. It is undoubtedly one of the most ubiquitous of all the *Penicillia*. *P. decumbens* is also abundant in nature and regularly occurs in soil dilution plates. *T. harzianum* is commonly isolated from soil, grains, pecans, paper and textiles. Although most of the screenings for biocontrol agents have been carried out involving the microorganism itself; it would be interesting to know if the metabolic broth where these fungi have been grown are also active. Mycoparasitism involving lytic enzymes has been already described as the mechanism of action of *Trichoderma* isolates in the biological control of commercially important plant pathogens [23]. *Trichoderma* employs a variety of antagonistic mechanisms for combating other fungi. The simplest one is probably competition for non-structurally-bound nutrients, however volatiles and soluble antifungal metabolites are also involved [24].

Table 1. Bactericidal activity of extracts: halos of inhibition (mm).

	Bacteria				
	Pseudomonas solanacearum	*Xanthomonas campestris*	*Agrobacterium tumefaciens*	*Eschericchia coli*	*Serratia marcescens*
Extract N*	100 µg extract/disk				
1	5	12	8	12	11
2	5	10	8	12	11
3	10	11	9	9	9
4	10	11	9	11	9
5	5	5	5	9	9
6	5	5	5	9	11
7	5	5	5	9	9
8	5	5	5	9	11
9	5	11	9	10	9
10	5	5	5	9	9
11	5	5	5	9	9
Control	5	5	5	5	5

* 1, 3, 5, 7, 9, extracted from cultures grown in potato dextrose broth of *T. harzianum, P. oxalicum* 1, *P. oxalicum* 2, *P. decumbens* and *P. oxalicum* 3, respectively.
2, 4, 6, 8, 10, extracted from cultures grown in Wickerham broth of *T. harzianum, P. oxalicum* 1, *P. oxalicum* 2, *P. decumbens* and *P. oxalicum* 3, respectively.
11, extracted from *P. oxalicum* 3 cultures grown in Wickerham broth plus maize flour.

Study of the antibiotic activity of extracts. Two different extracts were obtained from each fungus because they were grown on two different media (Wickerham (WB) and potato dextrose (PDB) broth). Results suggest that in general the extracts from potato dextrose broth have higher effectivity.

None of the extracts showed bactericidal capacity at a concentration of 50 µg/disk, however this changed with a 100 µg/disk concentration. Extracts 1, 2, 3, 4 and 9 showed the widest inhibitory effect (Table 1).

Referring to fungicidal activity (Table 2), results were generally better with a 100 µg extract/disk concentration. None of the extracts showed activity against *B. cynerea*. However, trichodex, a preparation from *T. harzianum* T39 controls diseases caused by *Botrytis cinerea* in greenhouse crops and vineyards [25]. This might indicate that there is an intraspecific difference, or that the antagonistic activity against *B. cinerea* is not due to the metabolite production but to the fungus itself and volatiles release.

The more active extracts were produced by *T. harzianum* (1 and 2) and *P. oxalicum* (3, 4 and 5). Among them, to date, only *T. harzianum* has been investigate for antibiotic metabolites production. Then, culture extracts from nine antagonistic *Trichoderma* spp. have been tested

Table 2. Fungicidal activity of extracts: halos of inhibition (mm).

	Fungi									
	Aspergillus versicolor		*Penicillium griseofulvum*		*Aspergillus candidus*		*Curvularia trifolii*		*Botrytis cynerea*	
	µg extract/disk									
Extract N[a]	50	100	50	100	50	100	50	100	50	100
1	12	15	15	15	18	20	22	26	5	5
2	7	7	10	10	15	15	7	7	5	5
3	17	19	5	5	5	10	20	27	5	5
4	12	17	5	5	5	10	11	20	5	5
5	5	5	5	5	5	5	22	27	5	5
6	5	5	5	5	5	5	5	5	5	5
7	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5
10	5	5	5	5	5	5	5	5	5	5
11	5	5	5	5	5	5	5	5	5	5
Control	5	5	5	5	5	5	5	5	5	5

* 1, 3, 5, 7, 9, extracted from cultures grown in potato dextrose broth of *T. harzianum, P. oxalicum* 1, *P. oxalicum* 2, *P. decumbens* and *P. oxalicum* 3, respectively.
2, 4, 6, 8, 10, extracted from cultures grown in Wickerham broth of *T. harzianum, P. oxalicum* 1, *P. oxalicum* 2, *P. decumbens* and *P. oxalicum* 3, respectively.
11, extracted from *P. oxalicum* 3 cultures grown in Wickerham broth plus maize flour.

Table 3. Percentages of mortality induced by fungal extracts on *Oncopeltus fasciatus* individuals.

N. extract*	1	2	3	4	5	6	7	8	9	10	11	C
After 3 days treatment	66.6	40	0	66.6	13.3	60	13.3	20	46.7	80	73.3	0
After 7 days treatment	73.3	73.3	60	100	46.6	60	33.3	46.7	46.7	86.7	80	0
After 14 days treatment	80.3	80	60	100	46.6	60	33.3	46.7	46.7	86.7	80	0
After 3 days treatment+ 3 days normal conditions	-	-	60	100	-	-	-	-	13.3	-	-	0

* 1, 3, 5, 7, 9, extracted from cultures grown in potato dextrose broth of *T. harzianum, P. oxalicum* 1, *P. oxalicum* 2, *P. decumbens* and *P. oxalicum* 3, respectively.
2, 4, 6, 8, 10, extracted from cultures grown in Wickerham broth of *T. harzianum, P. oxalicum* 1, *P. oxalicum* 2, *P. decumbens* and *P. oxalicum* 3, respectively.
11, extracted from *P. oxalicum* 3 cultures grown in Wickerham broth plus maize flour.
C = control

on *Geotrichum candidum* for antibiotic activity, and all produced antifungal metabolites [26]. These antifungal metabolites could only be detected after conidiogenesis [24].

One of these substances has been identified as 3-(2-hydroxypropyl)-4-(2-hexadienyl)-2(5-hydroxyl)-furanone, a new natural product [27]. Trichodermin, a commercial product obtained from *T. koningii* has been used for control of root rots on greenhouse-grown tomato and cucumber [28], for control of *Curvularia* leaf spot of yam [29], and for treatment of *Capsicum* plants against *Verticillum dahliae* [30].

Very different activities were shown by *P. oxalicum* isolates, which is in accordance with the great variability found among the isolates of the different *Penicillium* species in toxigenic capacity of the secondary substances or metabolites excreted [31]. *P. oxalicum* has been reported to be a biocontrol agent for *Fusarium oxysporum* f. sp. *lycopersici* [32]. Leaf spot severity (*Cercospora canescens*) on *Vigna mungo* was reduced by spraying with *P. oxalicum* before inoculation of *C. canescens* [33]. A study by Pandey *et al*. [11] suggested that volatiles produced from *P. oxalicum* inhibited the growth of *Glomerella cingulata*, while volatiles from *T. harzianum* inhibited the growth of *Pestalotia psidii*. Application of a spore suspension of each test fungus inhibited lesion development of guava leaves caused by the test pathogens in vitro. However, metabolite extracts from this species have not been tested for inhibitory activity.

P. decumbens has been investigated for the obtention of enzymes industrially applied such as cellullases [34] and naringinases [35], but not for antibiotic metabolites.

On the whole, extracts from one isolate of *P. oxalicum* Corrie and Thom, presented the maximum activity both in terms of bactericidal and fungicidal activity, higher than the well-known *Trichoderma* species. In general, PDB was a more suitable medium for the production of antibiotic compounds, than WB was.

Study of the insecticidal activity. Table 3 shows the results obtained in the insecticidal activity test with the assayed extracts. In general, oppositely to previous section, higher activities were shown by extracts from cultures on WB than those on PDB. High mortalities were reported after three days of contact, moreover, mortality increased from three to seven days, but then remained constant, with no differences between seven and 14 days of treatment. Extract 4 led to a 100% mortality, while extracts 1, 2, 10 and 11 provoked the death of 80% of the individuals, and even a higher percentage. These effective extracts came from *P. oxalicum* and *T. rifai* cultures. Extracts from cultures of *Penicillium funiculosum* have been reported to induce 80-100% mortality of the same insect [3].

Penicillium and *Trichoderma* species have been isolated from *Glossina pallidipes*, however, their were only mildly pathogenic for adult *Glossina*, compared to bacteria isolates, and were not pathogenic for pupae [8]. By contrast, fungal genera associated with *Stromatium fulvum* larvae were isolated and pathogenicity test done; *Trichoderma* and *Penicillium* were among the genera recommended as biological control agents for *S. fulvum* larvae [7].

A laboratory method was described by Jassim *et al.* (1990) for the rearing of larvae of *Scolytus multistriatus*

and *S. scolytus* on an artificial diet following exposure to cultures of microorganisms. In control colonies, the natural mortality for both species was 21.2 and 17.6%, respectively. Inoculation of *Trichoderma harzianum* caused more than 80% larval mortality.

Referring to retarded activity, it was only shown by extracts 3 and 4 (*P. oxalicum*). Insects treated with extract 3 showed an initial resistance (0% mortality after three days), but once the treatment finished mortality increased up to 60%.

P. oxalicum has been reported to be a pathogenic fungus of the aphid *Ceratovacuna lanigera*, an insect pest on sugarcane [29].

Out of the five isolates tested, *P. decumbens* showed the least bactericidal, fungicidal and insecticidal activity.

The results showed in this paper are a preliminary study to find species with capacity to control harmful fungi and insects. In a future other studies will be necessary to know the possibility to use these fungi as biological pesticides.

References

1. Yamaguchi I. Natural products as agrochemicals and leads. Extended Sumary SCI Pesticides Group Symposium. Novel approaches in agrochemical research III. Pestic Sci 1992; 35: 391-392.
2. Dybas RA. Avermectins their chemistry and pesticidal activities. En: Miyamoto J, Keaney PC (Eds.) Pesticide chemistry. New York, Pergamon Press, 1984: 83-90.
3. Santamarina MP, Jiménez M, Sanchis V, García F, Hernández E. A strain of *Penicillium funiculosum* Thom with activity against *Panonychus ulmi* Toch (Acar., Tetranychida). J Appl Ent 1987; 103: 471-476.
4. Shields JK, Atwell EA. Effect of a mold, *Trichoderma viride*, on decay by four storage fungi. For Prod J 1963; 13: 262-265.
5. Turhan G, Gossmann F. Antagonistic activity of five *Myrothecium* species against fungi and bacteria in vitro. J Phytopathology 1994; 140: 97-113.
6. Fang HM, Tan SM. Pathogenic fungi of several insect pests on sugarcane. Microbiology 1986; 13: 97-100.
7. Hassan FA, Omran TA, Badran OA, Gaaboub EA. Studies on the wood-borer *Stromatium fulvum* Vill. (*Col., Cerambycidae*) infesting *Casuarina glauca (Casuarinaceae)*. II. Entomogenous fungi associated with *Stromatium* larvae. Anzeiger fur Schadlingskunde, Pflanzenchutz, Umweltschutz 1988; 61: 8-11.
8. Kaaya GP, Okech MA. Microorganisms associated with tsese in nature: preliminary results on isolation, identification and pathogenicity. Insect Science and its Application 1990; 11: 443-448.
9. Larrondo J, Calvo MA. The antimicrobial capacity of *Penicillium* and *Aspergillus* strains isolated from vineyards soils. Microbios Letters 1990; 44: 77-81.
10. Molina GC, Davide RG. Evaluation of microbial extracts for nematicidal activity against plant parasitic nematodes *Meloidogyne incognita* and *Radopholus similis.* Philippine Agriculturist 1986; 69: 173-186.
11. Pandey RR, Arora DK, Dubey RC. Antagonistic interactions between fungal pathogens and phyllophane fungi of guava. Mycopathologia 1993; 124: 31-39.
12. Whipps JM. Advances in biological control in protected crops. Brighton Crop Protection Conference, Pests and Diseases. Farnham, UK, British Crop Protection Council, 1994: 1259-1264.
13. Lewis JA, Papavizas GC. Biocontrol of plant diseases: the approach for tomorrow. Crop Protection 1991; 10: 95-105.
14. Jijakli MH, Lepoivre P. Use of biological pesticides for the protection of apples in storage. Fruit Belge 1995; 63: 83-88.
15. Marchetti R, Nipoti P, Ercole ND, Guerzoni ME. Competition at atmospheric level as biocontrol mechanism in *Trichoderma* spp. Petria 1992; 2: 137-147.
16. Jiménez M, Santamarina MP, Sanchis V, Hernández E. Investigación de la micoflora y micotoxinas de cereales almacenados. Microbiologie - Aliments – Nutrition 1987; 5: 105-109.
17. Huerta T, Sanchis V, Hernández-Haba J, Hernández E. Enzymatic activities and antimicrobial effects of *Aspergillus* and *Penicillium* strains isolated from Spanish dry-cured ham. Microbiologie - Aliments – Nutrition 1987; 5: 289-294.
18. Harman GE, Backman PA, Turner JT, *et al*. Symposium: biocontrol and biotechnological methods for controlling cotton pests. En: Brown JM, Richter DA (Eds) Proceedings of the Beltwide cotton production research conferences. Memphis, Tennessee, USA. 1989: 15-20.
19. Lartey RT, Curl EA, Peterson CM. Interactions of mycophagous collembola and biological control fungi in the supression of *Rhizoctonia solani.* Soil Biol Biochem 1994; 26: 1-88
20. Latunde AO. The use of *Trichoderma koningii* in the control of web blight disease caused by *Rhizoctonia solani* in the foliage of cowpea (*Vigna unguiculata*). J Phytopathol 1991; 133: 247-254.
21. Canessa EA, Morrell JJ. Effect of *Trichoderma harzianum* on induction of laccase by *Trametes versicolor* on ponderosa pine sapwood. Document International Research Group on Wood Preservation. Stockholm: IRG Secretariat, 1996.
22. Dal Bello GM, Monaco CI, Chaves AR. Efecto de los metabolitos volátiles de *Trichoderma hamatum* sobre el crecimiento de hongos fitopatógenos procedentes del suelo. Rev Iberoam Micol 1997; 14: 131-134.
23. Bruce A, Srinivasan V, Staines HJ, Highley TL. Chitinase and laminarinase production in liquid culture by *Trichoderma* spp. and their role in biocontrol of wood decay fungi. Int Biodet Biodeg 1995; 35:337-353.
24. Horvath EM, Bürgel JL, Messner K. The production of soluble antifungal metabolites by the biocontrol fungus *Trichoderma harzianum* in connection with the formation of conidiospores. Material and Organismen 1995; 29: 1-14.
25. Elad Y, Shtienberg AN. *Trichoderma harzianum* T39 integrated with fungicides; improved biocontrol of grey mould. Brighton Crop Protection Conference, Pest and Diseases 1994; 3: 1109-1114.
26. Altomare C, Bottalico A. A toxicological approach to the biocontrol of plant pathogens by *Trichoderma*. Bulletin OILB/SROP 1992; 15: 88-90.
27. Ordentlich A, Wiesman Z, Gottlieb HE, Cojocaru M, Chet I. Inhibitory furanone produced by the biocontrol agent *Trichoderma harzianum*. Phytochemistry 1992; 31: 485-486.
28. Stirmanova NI. New commercial forms of fungal preparations. Zashchita Rastenii Moskva 1994; 9:7-8.
29. Michereff SJ, Silveira NSS, Reis A, Mariano LR. Greenhouse screening of *Trichoderma* isolates for control of *Curvularia* leaf spot of yam. Mycopathologia 1995; 130: 103-108.
30. Georgieva O. Antagonistic characteristics of *Trichoderma koningii* towards *Verticillium dahliae* on pepper. Bulletin OILB/SROP 1988; 15: 18-20.
31. Jiménez M, Sanchis V, Mateo R, Hernández E. *Penicillium* in pre-harvest corn in Valencia (Spain) II. Study of the enzymatic and toxigenic capacities of the species. Mycopathologia 1986; 96: 13-18.
32. De Cal A, Pascual S, Melgarejo P. Involvement of resistance induction by *Penicillium oxalicum* in the biocontrol of tomato wilt. Plant Pathology 1997; 46: 72-79.
33. Rao PB, Mallaiah KV. Effect of phyllophane fungi on the leaf spot pathogen *Cercospora canescens*. Indian J Microbiol 1988; 28: 103-107.
34. Zhao X, Qu YB, Gao DJ. Acceleration of ethanol production from paper mill waste fiber by supplementation with beta-glucosidase. Enzyme Microbial Technol 1993; 15: 62-65.
35. Hsieh PC, Tsen HY. Purification and characterization for alpha-rhamnosidase from two fungal naringinase preparations. J Chinese Agric Chem Soc 1991; 29: 61-73.