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Antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfsii*: screening of efficient isolates from Morocco soils for biological control

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Summary. Seventy *Trichoderma* spp. isolates collected from different regions of Morocco were tested for their capacity to inhibit *in vitro* mycelial growth of *Sclerotium rolfsii*, and for their effect on the viability of *S. rolfsii* sclerotia in the soil. The *Trichoderma* spp. isolates inhibited mycelial growth of *S. rolfsii* to various degrees, with 52% of isolates expressing an average inhibition, varying between 45 and 55%. The effect on the viability of sclerotia in the soil also varied between isolates of *Trichoderma*, with the majority (84%) having a slight effect. A group of twenty isolates identified as *Trichoderma harzianum* when tested in sterilized soil, significantly reduced sclerotial viability though not in natural soil. Four of these isolates (Nz, Kb2, Kb3 and Kf1) showed good antagonistic activity against *S. rolfsii* and were also highly competitive in natural soil. These isolates would therefore be candidates for development in biological control program.

Key words: sugar beet, antagonism, sclerotia.

Introduction

In Morocco, the root rot pathogen *Sclerotium rolfsii* causes damage to sugar beet mainly in the irrigated region of Doukkala. The pathogen attacks sugar beet tubers a few weeks prior to harvest, causing up to 50% losses in crop yield and quality (Fidah, 1995; Ezzahiri, 2000). The pathogen survives in the soil in the form of sclerotia and the disease incidence in a sugar beet field was found to be correlated with the density of these fungal structures in the soil (Fidah, 1995).

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Although, many fungicides are effective against *S. rolfsii*, their use in practice is not justified economically or environmentally, since treatments must be repeated at least three times without total control being guaranteed. Efficient control of *Sclerotium* root rot of sugar beet thus relies on chemical control integrated with preventive practices (rotation, deep plowing, soil solarization aimed at reducing the survival capacity of sclerotia in the soil. The effect of these practices could be enhanced by antagonists such as *Trichoderma* spp. However, their use in practice requires them to be effective against the target pathogens (James *et al*., 1993), and they also need to be adapted to the environment in which they are to be utilized (Monte, 2001; Howel, 2003). Thus, it is necessary to screen locally adapted populations of antagonists

for isolates that will prevent and control *S. rolfsii* (Howel, 2003).

The most common method to select optimal antagonists is with a dual culture test in Petri dishes (Bell *et al*., 1982; Henis, 1984; Velasquez and Pineda, 1994; Ouazzani *et al*., 1995). For antagonism against *S. rolfsii*, additional tests are also desirable, such as those to determine the effect of the antagonists on the viability of the sclerotia (Davet, 1986; Jones and Stewart, 1995; Cundon *et al*., 2000) and the saprophytic competitive ability of the antagonists (Davet, 1986).

In this study, as part of a program to develop biological control of *S. rolfsii*, 70 *Trichoderma* spp. isolates collected from various regions of Morocco were screened for antagonist effectiveness. Characteristics examined were: *in vitro* mycelial growth inhibition, sclerotial viability in the soil, and competitive saprophytic ability.

Materials and methods

Production of sclerotia

Isolate Sr1 of *S. rolfsii* used in this study was obtained from an infected tuber of sugar beet collected from the Doukkala region in Morocco. For massive production of sclerotia, we utilized a mixture of sand (50 g) and wheat bran (20 g). The substrate was homogenized and placed in 250 ml flasks, then autoclaved at 120°C for 45 min. To each flask, 20 ml of 1%, autoclaved water agar was added. Five mycelial disks (5 mm diam.), obtained from 7-day-old potato dextrose agar (PDA) cultures of *S. rolfsii,* were transferred to each flask. Cultures were incubated at 30°C in the dark for 5 weeks. The sclerotia that formed were harvested, dried and stored at 4°C until use.

Soil sampling, isolation and production of *Trichoderma* inoculum

Fifty soil samples were collected in the Doukkala region, from both irrigated and non irrigated fields (Table 1). Twenty further soil samples were taken from 10 other agro-ecological regions in Morocco (Table 2). The soil samples were taken at 10 cm depth, dried for 2 days at 28°C, sieved and kept at 10°C until used.

Trichoderma spp. were isolated from the soil samples using the soil dilution technique. The sieved soil samples (10 g) were shaken in 90 ml of sterile distilled water (SDW) for 10 min. The resulting soil suspensions were diluted to 10^{-4}. Aliquots (1 ml) of the last dilution were added to Petri dishes containing 20 ml of PDA and 0.5 g l^{-1} of chloramphenicol and the dishes were incubated at 28°C. The resulting *Trichoderma* colonies were purified on PDA amended with chloramphenicol (0.5 g l^{-1}) and a code was assigned to each isolate (Tables 1 and 2). The isolates were stored at 4°C until use.

Among the 70 isolates collected, 20 were identified by Roquebert (International Museum of Natural Histories, France) as *Trichoderma harzianum* (Tables 1 and 2).

The inoculum of *Trichoderma* isolates was prepared using a mixture of 200 g sand, 6 g oat meal and 30 ml SDW.

The mixture was homogenized, placed in 250 ml flasks and autoclaved at 120°C for 45 min. Flasks were inoculated with 5 ml each of a spore suspension of *Trichoderma* spp. (10^7 conidia ml^{-1}) from 20-day-old PDA cultures. After incubation at 28°C for 4 weeks in the dark the infected substrate was mixed with 100 ml SDW. The suspension was then shaken for 10 min on a wrist-action shaker and adjusted to a concentration of about 10^7 conidia (plus mycelium) for each *Trichoderma* isolate.

Soil used for test

The soil used to test the effect of *Trichoderma* isolates on sclerotial viability had the following physico-chemical and biological characteristics: pH 8.08; total P, 0.032%; total N, 0.37%; organic C, 0.85%; electrical conductivity, 413.5 µs; *Fusarium*, 3.26 10^4 cfu g^{-1} of soil; *Aspergillus*, 10.6 10^4 cfu g^{-1} of soil; *Penicillium*, 2.9 10^4 cfu g^{-1} of soil; Démathiées, 2.4 10^4 cfu g^{-1} of soil; Mucorales, 0.3 10^4 cfu g^{-1} of soil; *Trichoderma* spp., 1 10^4 cfu g^{-1} of soil.

The soil was collected from Doukkala region, grounded, passed through a 0.4 mm diameter sieve and dried at 28°C for 48 h.

Effect of *Trichoderma* isolates on sclerotial viability in the soil

The antagonistic effect of *Trichoderma* isolates against *S. rolfsii* was tested following Artigues and Davet (1984). Twenty sclerotia of *S. rolfsii* were added to 25 g of soil in a plastic bag, and to this was added a conidial suspension of *Trichoderma* at a concentration of 10^7 conidia g^{-1} soil. The soil was adjusted to 70% moisture holding capacity. All

Table 1. Antagonistic action of *Trichoderma* isolates, originating from Doukkala, on *S. rolfsii*.

Isolate	Locality	Mortality [a] (%)	Growth inhibition[b] (%)
Kb2*	Khemis Zemamra (n.irr)[c]	71±8.4	61±11.5
Kf1*	Khemis Zemamra (irr)	49±9.5	53±9.5
Kb12*	Sidi Bennour (n.irr)	46±12.7	52±10.5
Ke2	Sidi Bennour (irr)	44±6.5	57±4.4
Kb3*	Khemis Zemamra (n.irr)	43±13.5	52±5.7
P37	Khemis Zemamra (irr)	43±7.7	34±5.2
Kd8*	Sidi Bennour (irr)	42±4.7	35±12.4
Kf3*	Khemis Zemamra (irr)	39±3.7	52±8.7
Kc3	Khemis Zemamra (irr)	34±11.6	41±1.50
P63	Khemis Zemamra (irr)	34±8.4	70±11.4
P32	Khemis Zemamra (irr)	33±12.6	46±5.5
P35	Khemis Zemamra (irr)	33±7.3	5±8.7
Kb6*	Khemis Zemamra (n.irr)	32±11.4	48±7.8
1ze	Khemis Zemamra (irr)	31±8.2	52±7.5
Ke3	Khemis Zemamra (irr)	31±9.7	52±9.3
P34	Khemis Zemamra (irr)	31±5.6	37±5.4
Kb5*	Sidi Bennour (irr)	30±10.8	54±5.7
Ka2	Sidi Bennour (irr)	28±6.5	48±9.5
P31	Khemis Zemamra (irr)	26±8.3	50±5.5
Kd5	Sidi Bennour (irr)	25±9.2	47±8.5
P61	Khemis Zemamra (irr)	25±4.7	49±4.4
K16	Sidi Bennour (irr)	24±3.5	56±4.3
Ka1	Sidi Bennour (irr)	24±6.4	23±5.8
P36*	Khemis Zemamra (irr)	24±5.6	41±5.6
B35*	Sidi Bennour (n.irr)	22±7.2	49±4.5
P33	Khemis Zemamra (irr)	22±4.7	50±7.3
Kc1	Khemis Zemamra (irr)	21±7.5	40±11.5
Kc4	Khemis Zemamra (irr)	21±5.5	56±8.7
Ke1	Khemis Zemamra (irr)	21±3.4	46±7.5
2Ze	Khemis Zemamra (irr)	20±5.6	46±5.2
Kb13	Khemis Zemamra (n.irr)	20±8.5	54±5.8
A3sb	Sidi Bennour (irr)	19±7.7	50±4.5
Kb7	Khemis Zemamra (n.irr)	19±5.7	50±3.6
Kd2	Sidi Bennour (irr)	19±6.5	59±5.8
P62	Khemis Zemamra (irr)	19±5.6	36±6.7
Kc2	Khemis Zemamra (irr)	17±3.6	48±8.8
Kd3	Sidi Bennour (irr)	17±5.5	53±8.4
P81	Khemis Zemamra (irr)	17±5.6	23±7.4
3Ze	Khemis Zemamra (irr)	16±8.7	39±11.2
Kb8*	Khemis Zemamra (irr)	16±9.2	60±6.3
Kf2	Khemis Zemamra (irr)	16±4.4	52±3.5
Kd9	Sidi Bennour (irr)	15±8.5	49±2.4
Kb4*	Khemis Zemamra (n.irr)	14±8.4	45±8.6
P80	Khemis Zemamra (irr)	13±9.5	50±8.8
Kb11	Sidi Bennour (n.irr)	12±5.4	55±7.5
Kd1*	Sidi Bennour (irr)	12±4.6	58±4.7
Kd4*	Sidi Bennour (irr)	11±3.4	12±4.8
Kd6	Sidi Bennour (irr)	11±4.5	28±5.7
P82	Khemis Zemamra (irr)	11±4	48±8.5
Kb1*	Sidi Bennour (n.irr)	10±3	20±8.5

[a] Percentage of dead sclerotia in natural soil after four week of incubation at 30°C.
[b] Percentage of mycelial growth inhibition of *S. rolfsii* in dual culture on PDA in Petri dishes after 48 hours at 30°C.
*, isolates identified as *T. harzianum*.
[c] (irr), irrigated field; (n.irr), non irrigated field.

Table 2. Antagonistic action of *Trichoderma* isolates originating from other region of Morocco on *S. rolfsii*.

Isolate	Region	Locality	Mortality[a] (%)	Growth inhibition[b] (%)
Nz*	Tadla	Field of sugar beet	65±11.2	61±8.8
P5af-1	Sais	Field of cereals	45±7.5	58±7.5
Oliv2	Sraghna	Field of cereals	30±8.5	34±5.8
Jf	Haouz	Garden	29±6.6	53±8.8
P1af-2	Saïs	Field of cereals	28±9.5	51±4.6
Oliv2-1	Sraghna	Field of cereals	26±5.3	29±3.6
P2af-1	Saïs	Field of cereals	26±8.5	61±7.8
R1*	Zaer	Forest of *Pinus halepensis*	26±4.7	55±5.7
Mh*	Haouz	High Atlas (forest of *Pinus halepensis*)	21±7.8	52±7.8
Ch1	Chiadma	Forest of *Argania spinosa*	20±8.5	79±4.9
R3	Zaer	Experimental garden	20±4.8	50±8.6
Ch1*	Chiadma	Forest of *Argania spinosa*	20±3.7	79±9.6
R4	Zaer	Forest of Eucalyptus	20±2.8	42±5.6
S2*	Chiadma	Forest of *Argania spinosa*	19±3.2	64±10.8
M1b	Haouz	High Atlas (forest of *Cupressus atlantica*)	18±5.5	54±11.2
Ag2	Sous	Forest of *Argania spinosa*	17±4.7	56±6.8
Th*	Gharb	Laboratory	17±3.2	54±8.6
P7-af1	Sais	Field of cereals	16±5.2	62±7.5
Ag1	Agadir	Forest of *Argania spinosa*	13±4.4	31±5.6
M2	Haouz	High Atlas (forest of *Pinus halepensis*)	13±5.6	31±9.3

[a] See Table 1.
[b] See Table 1.
*, isolates identified as *T. harzianum*.

treatments were replicated six times. After 10 days of incubation at 30°C, the sclerotia were removed from the soil, disinfected with 1% sodium hypochlorite for 3 min and their viability determined.

The 20 isolates identified as *T. harzianum* were compared for their antagonistic activity against sclerotia of *S. rolfsii* also in the sterilized soil (45 min at 120°C in the autoclave).

Dual culture of *Trichoderma* isolates and *S. rolfsii*

Mycelial disks (5 mm diameter) taken from the margins of 6-day-old colonies of *Trichoderma* and *S. rolfsii* were transferred to Petri dishes containing 20 ml PDA. One isolate of *Trichoderma* and one of *S. rolfsii* were placed simultaneously on opposite sides of each dish, 5 cm apart. The control was prepared with only *S. rolfsii*. Six Petri dishes were used for each treatment. Mycelial growth of *S. rolfsii* was assessed by measuring the colony diameter in two perpendicular directions after 48 h of incubation at 30°C. Percent inhibition (I) of mycelial growth of *S. rolfsii* in the presence of *Trichoderma* spp. was determined according to the method of Camporota (1985):

$$I = (C_1\text{-}C_2/C_1) \times 100$$

where C_1 is the linear growth of *S. rolfsii* in control Petri dishes, and C_2 is the linear growth of *S. rolfsii* in dual culture with the *Trichoderma* isolate.

Saprophytic competitive activity of *T. harzianum* isolates

The competitive saprophytic activity of *T. harzianum* isolates was determined with the agar disk method of Davet and Camporota (1986). Twelve g of natural soil was mixed with each of the *T. harzianum* isolates to give 10^7 conidia g^{-1} of soil. The inoculated soil was transferred to a Petri dish to which 20 ml of autoclaved water-agar was added. After solidification, four disks (7 mm in diameter) of the medium (soil-agar mixture) were placed in the Petri dishes containing PDA at the four compass points. For each *T. harzianum* isolate, the treatment was replicated five times.

Growth of *T. harzianum* isolates in the Petri dishes was scored on a 4-point scale score, where 0, no growth of *T. harzianum* around the agar disk; 4, *T. harzianum* completely overgrows the agar disk. The saprophytic competitive activity (C) of each *T. harzianum* isolate was the sum of the scores

recorded in all Petri dishes transformed to a percentage.

Results

Dual-culture test

The 50 *Trichoderma* isolates from the Doukkala region varied in their antagonism against *S. rolfsii*, with inhibition percentages (I) ranging from 5±8.7% to 70±11.4% (Table 1). However, with a majority of isolates (52%) inhibition varied between 45 and 55%, so that both weak inhibitors (I <45%) and strong inhibitors (I>55%) of *S. rolfsii* represented only a small proportion of the entire group of isolates: 30 and 18% respectively. The distribution of the I had a median value of 49 and a mode of 52 (Fig. 1a). Growth inhibition caused by the 20 isolates from regions of Morocco other than Doukkala varied between 29±3.6% and 79±9.6%

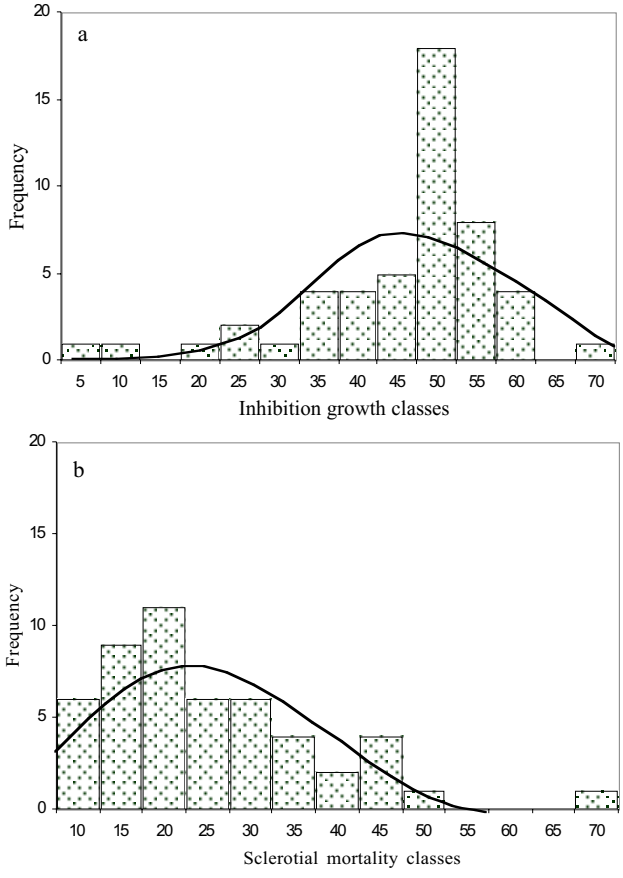

Fig. 1. Distribution of the frequency of *T. harzianum* isolates from Doukkalla region, according to: a, mycelial growth inhibition of *S. rolfsii* on PDA dishes; b, antagonistic action on sclerotia of *S. rolfsii* in the soil.

but most isolates (70%) showed considerable inhibition of mycelial growth (I values greater than 50%) (Table 2).

Antagonistic activity of *T. harzianum* on sclerotia in natural soil

Almost all the *Trichoderma* spp. isolates from the Doukkala region reduced sclerotia viability, but only 3 isolates reduced mortality of sclerotia by more than 45%: these were Kb2, Kf1 and Kb12, comprising 6% of all isolates found. The percentage of sclerotial mortality (M) fluctuated between 10.2±3% and 70.8±8.4% (Table 1) with the majority of *Trichoderma* isolates characterized by weak antagonistic action. Twenty one isolates (42%) caused mortality between 10 and 20%; 13 (26%) between 21 and 30%; 9 (18%) between 31 and 40% and 6 (12%) between 41 and 50%. The remaining isolate (Kb2) caused a mortality of 71±8.4%. The median value of the M frequency distribution was 21.5 and the mode value was 19 (Fig. 1b).

Most *Trichoderma* isolates from other regions of Morocco also exhibited a weak antagonism, with a mortality rate not exceeding 45%. Only one isolate (Nz) had rather good antagonistic activity (M = 65±11.2).

Antagonistic activity of *T. harzianum* on sclerotia in sterilized soil

In sterilized soil, the antagonistic activity of *T. harzianum* isolates was clearly more than that in natural soil (Table 3). For example, the sclerotia mortality of isolates Kb1, Kb4, P36, and S2, was 60.6, 80.5, 71.7 and 80.6% in sterilized soil respectively, but only 10, 14, 24 and 19% in natural soil. In sterilized soil, the mortality of sclerotia caused by the different isolates varied between 50.5 and 92.6%; in natural soil, it ranged from 10.2 to 70.8%.

Saprophytic competitive activity of *T. harzianum* isolates

Trichoderma harzianum isolates varied in saprophytic competitive activity as shown by their C values, which varied between 15 and 100% (Table 3). The most competitive isolates were those with C values between 90 and 100%, which were Kf1, Kb3, Kb2 and Nz. Isolates Kd4, P63, Kf3 and Kb12 showed average competitiveness, with C values between 55 and 75%. Non-competitive isolates (C values less than 55%) were R1, Kb13, Kd2, Kb1, S2, Mh, Jf, Th, Kb8, Kb5, Kb4 and P36.

Table 3. Antagonistic action of *T. harzianum* isolates on sclerotia of *S. rolfsii* in natural soil and in sterilized soil, their effect on mycelial growth of *S. rolfsii* after 72 hours of incubation at 30°C and their saprophytic competitive ability on PDA in Petri dishes.

Isolate	Mortality of sclerotia		Growth inhibition (%)	Competitiveness (%)
	in sterilized soil (%)	in natural soil (%)		
Nz	66±7.8	65±8.8	61±11.2	100
Kb2	68±5.8	71±8.4	61±11.5	100
Kb3	73±8.5	43±13.5	52±5.7	100
Kf1	68±11.3	49±9.5	53±9.5	90
P63	73±14.5	34±8.4	70±11.4	70
Kf3	92+9.6	39±3.7	52±8.7	70
Kb12	81±10.5	46±12.7	52±10.5	70
R1	76±8.7	26±4.7	55±5.7	50
Kb13	77±7.9	20±8.5	54±5.8	50
Jf	60±8.8	29±6.6	53±8.8	50
Th	50±8.7	17±4.7	54±8.6	45
Kb8	62±5.4	16±9.2	60±6.3	30
Kd4	50±11.2	11±3.4	12±4.8	75
Kd2	77±5.6	19±6.5	52±5.8	30
Kb5	57±10.1	30±10.8	54±5.7	25
Kb1	60±6.6	10±3	20±8.5	55
Kb4	80±6.5	14±8.4	45±8.6	15
P36	71±5.5	24±5.6	41±5.6	15
S2	80±11.2	19±3.2	64±10.8	15
Mh	53±8.7	21±7.8	52±7.8	15
Mean	68.75	30.15	51.2	53.5
SD	11.36	17.24	13.66	29.96

Comparison of the inhibitory activity of different *Trichoderma* isolates

Comparison of mycelial growth inhibition by *Trichoderma* and *S. rolfsii* sclerotial viability in natural soil revealed only a weak relationship between these two variables (Table 1). Some *Trichoderma* isolates that were good inhibitors of mycelial growth on PDA dishes, were only weak inhibitors in the soil. This was the case with isolates S2, Kb8, Kd2 and P63. However, two points deserve to be made here: 1. isolates with weak inhibitory activity *in vitro* were also weak inhibitors in natural soil, for instance, Kd4, Kb1, Ka1, Kd6, P81, M2, Ag1 and Oliv2-1; 2. Isolates that were good inhibitors in the soil were also good inhibitors *in vitro*, as in the case of isolates Nz, Kb2, Kb3, Kf1, Kb12.

The antagonistic action of *T. harzianum* isolates against sclerotia of *S. rolfsii* was fairly well related to competitive saprophytic capacity (Table 3). Saprophytic competitiveness correlated well with percent sclerotial mortality in natural soil, with r equal to 0.7 (Fig. 2). Therefore there was no correlation between saprophytic competitive capacity and antagonistic action in sterilized soil or *in vitro*: here the correlation coefficient was very low (r=0.08 in sterilized soil and 0.07 *in vitro*) (Fig. 2). Antagonistic activity in sterilized soil also correlated weakly with inhibitory action on PDA with r=0.35 (Fig. 2).

The isolates which were competitive and effective *in vitro* on PDA were also effective in natural soil, whereas the isolates that were non-competitive and/or non efficient *in vitro* were weakly efficient in the soil. The activity of an isolate in the soil therefore reflected both its *in vitro* activity and its competitiveness. Antagonist effectiveness in the soil was thus highly correlated with the sum of the inhibition rating and the competitiveness, expressed by the factor I + C (r=0.85).

Four of the isolates, Nz from Beni Mellal, Kb2

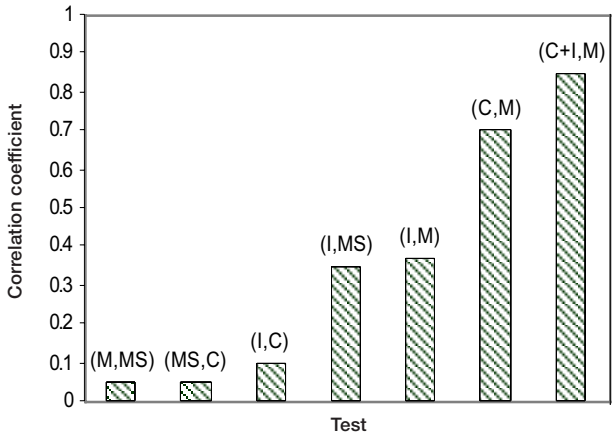

Fig. 2. Correlation between the effects of *T. harzianum* isolates against *S. rolfsii* obtained by different tests: percentage of dead sclerotia in natural soil (M), in sterilized soil (Ms), percentage of mycelial growth inhibition in Petri dishes (I) and their saprophytic competitiveness (C).

and Kb3 from a non-irrigated area, and Kf1 from an irrigated area of the Doukkala region, gave good results in the three tests: they were competitive, effective *in vitro* and had good inhibition of sclerotia in the soil.

Discussion

The majority of the 50 *Trichoderma* spp. isolates originating from the Doukkala region showed weak antagonistic activity against *S. rolfsii*, with a sclerotial destruction rate lower than 45%. This finding, together with the low density of *Trichoderma* in the soils of Doukkala region (Louali, 1998) may explain the high incidence of disease due to *S. rolfsii* in this region.

The *T. harzianum* isolates from the same locations in the Doukkala region varied greatly in their antagonistic effectiveness. Wide variation in the antagonism of a population of *T. harzianum* was reported by Davet (1986). Isolate antagonism was not correlated with particular site conditions. Isolates with good and poor antagonism came indifferently from irrigated and non-irrigated areas.

The dual-culture test showed that isolates with average antagonistic effectiveness against *S. rolfsii* were dominant. In a similar test, Bell *et al.* (1982) also found that most of a group of 33 *T. harzianum* isolates expressed average antagonism against *S. rolfsii*. They found as well that most isolates were weakly antagonistic against other pathogens such as *Phytophthora* spp. It seems thus that the dominance of isolates with average antagonist effectiveness found in this study was mainly linked to the confrontation *Trichoderma-S. rolfsii*.

The weak correlation between the soil tests and the *in vitro* tests can be explained by interaction between physico-chemical and biological factors in the soil, which in turn influences the *Trichoderma* growth and antagonistic activity (Papavizas, 1985; James *et al.*, 1993; Khattabi and Ezzahiri, 2002; Kredics *et al.*, 2003). In an artificial, homogeneous environment such as a sterile PDA culture medium, these interactions would obviously be reduced. This would explain the weak dispersion of the results and their gathering around average values. Moreover, the mechanisms involved in antagonism, such as antibiosis, mycoparasitism and the relative importance of each differ depending on the test. The nature and nutritional factors of the test medium greatly influence the type of antagonism that is displayed (Howel, 2003).

Since *Trichoderma* antagonism was greater in sterilized soil than in natural soil, it seems probable that the microflora living in natural soil limited the antagonistic capacity of *Trichoderma*. This may have occurred in the Doukkala region., The effect of soil micro-organisms on *Trichoderma* was reported by some authors, who attributed it to rather complex mechanisms such as competition for nutritional sources (Papavizas, 1985; Davet, 1986; Adams, 1990). This competition is more marked in soils with a low organic matter content (Adams, 1990), and such soils are the rule in the Doukkala region (Soudi *et al.*, 2000).

In order to overcome the effect of the soil microflora on the antagonistic activity of *Trichoderma* isolates, some researchers have proposed to combine *T. harzianum* with other control methods such as fumigation, solarization (Elad and Chet, 1980; Hoynes *et al.*, 1999) and selective fungicide application for *T. harzianum* (Conway *et al.*, 1996; Khattabi *et al.*, 2001). Such combinations have given promising results and should be tested further under natural conditions in the Doukkala region.

In the competitiveness test *Trichoderma* isolates varied in their sensitivity to soil micro-organisms.

The agar disk method was a good way to assess how *Trichoderma* reacted to these micro-organisms. The competitiveness test gave consistent results, especially the fairly significant correlation between the competitiveness of *Trichoderma* and its antagonist effectiveness in natural soils. The method that we devised to assess *T. harzianum* competitiveness against other micro-organisms can also be used to determine a particular isolate's sensitivity to the inhibitory effects of bacteria. Kredics *et al*. (2003), stated that individual strains of *T. harzianum* vary in their sensitivity to bacteria.

The competitiveness of *T. harzianum* towards the soil microflora was unrelated to its antagonistic activity, which depended on precise mechanisms such as antibiosis and mycoparasitism. That is why the results of the *in vitro* test, which measured these mechanisms, bore no relation to the results of the competitiveness test (r=0.05). This test gave the same results as the comparison test in sterilized soil (r=0.05).

Thus, the antagonist effectiveness of *Trichoderma* isolates in natural soil seems to be indicated by two independent factors, its competitiveness and its *in vitro* activity. Therefore, non-competitive isolates, or those with low *in vitro* efficiency had only limited effectiveness in the soil, while competitive isolates that were also rather efficient *in vitro* gave good results in natural soil.

According to Henis (1984), antagonistic activity in the soil is a rather reliable test of the effectiveness of a strain since it is closely correlated with that strain's effect on disease incidence. However, a major drawback of such a test is that it is time consuming and sometimes gives inconsistent results (Davet, 1986). The dual-culture test adopted by many authors has the advantage of showing the variation in the antagonistic action of strains and the relative importance of the various antagonistic mechanisms (James *et al*., 1993). Nevertheless, the results with these tests are greatly influenced by the culture medium and nutritional factors (James *et al*., 1993; Howel, 2003), and they generally correlate badly with the strain effect on disease incidence (Henis, 1984; James *et al*., 1993; Howel, 2003). The determination of the competitiveness seems to fill up this gap to some extent.

The two tests enabled the effectiveness of *T. harzianum* strains to be reliably determined. They were a rapid and useful way to carry out the preliminary screening of potentially antagonistic *Trichoderma* isolates.

Among the seventy isolates tested, four (Nz, Kb2, Kb3 and Kf1) showed good antagonistic activity *in vitro* and were competitive in the soil. These are obvious candidates for further testing under natural conditions on a larger scale.

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