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# Effect of fungicides and *Trichoderma harzianum* on sclerotia of *Sclerotium rolfsii*

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**Summary.** The effect of three fungicides (benomyl, hymexazol, oxyquinoleine) on the viability of sclerotia of *Sclerotium rolfsii* was tested in natural and sterilized soils. A similar test was carried out in natural soil combining each of these fungicides with one of four isolates of *Trichoderma harzianum*. In addition, the mycelial growth of the *T. harzianum* isolates and *S. rolfsii* was monitored on agar media amended with these fungicides at three concentrations. Benomyl reduced the antagonistic ability of the *T. harzianum* isolates in the soil, oxyquinoleine yielded variable results, while hymexazol improved the antagonism of *T. harzianum* isolates. In an agar medium, benomyl inhibited all *T. harzianum* isolates, as did oxyquinoleine. By contrast, hymexazol had only a negligible effect on the growth of the antagonist.

**Key words**: *Sclerotium rolfsii*, *Trichoderma harzianum*, benomyl, hymexazol, oxyquinoleine.

## Introduction

*Sclerotium* root rot is a serious disease of sugar beet in the irrigated region of Doukkala (mid-west Morocco). Disease outbreaks occur mainly in July and August on mature roots, which are partially or completely destroyed. Disease incidence varies from traces to 50% and depends on the number of viable sclerotia in the soil (Fidah, 1995).

Control of *Sclerotium* root rot follows cultural, biological and chemical methods. Cultural practices rely on deep plowing and crop rotation. Chemical control is achieved with soil fumigants such as methyl-bromide and metam-sodium (Punja, 1985; Jenkins and Averre, 1986) or with the application of fungicides such as PCNB, carboxin, furmecyclox (Gurkin and Jenkins, 1985; Punja *et al.*, 1986), diniconazole and Flutolanil (Csinos, 1989). However, chemical control against *S. rolfsii* remains of limited value. It requires great amounts of fungicide and even then gives variable results depending on the season and the crop (Punja, 1985). For sugar beet, the exclusive use of fungicides for root rot control is not justified economically or ecologically.

Biological control using species of *Trichoderma* has been reported as effective in reducing sclerotial viability (Artigues and Davet, 1984) and controlling damping-off of beans caused by *S. rolfsii* in the greenhouse (Henis, 1984). However, the importance of the antagonistic effect of *Trichoderma*

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depends on several factors, such as the physico-chemical properties and microflora composition of the soil, and the competitive ability of the antagonists (Davet, 1986; Burpee, 1990; Adams, 1990).

In order to improve the effectiveness of *Trichoderma*, some authors have studied their effect in combination with other control methods, such as solar heating, soil fumigation (Elad *et al.*, 1980) or fungicide application (Chet *et al.*, 1979).

The aim of this study was to evaluate the combined effects of fungicides and *T. harzianum* isolates on the sclerotia of *S. rolfsii*.

## Materials and methods

### Fungal isolates

The isolate of *S. rolfsii* was originally derived from infested tuber of sugar beet. Sclerotia were produced on potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) by keeping cultures at 28°C for 4 weeks.

The four isolates of *T. harzianum* used were designated NZ (Tadla region), KF1, KB2 (Doukkala region) and ES (Chiadma region). They were selected for their effectiveness against *S. rolfsii* (Louali, 1998). Conidia of *T. harzianum* were produced by transferring agar disks (7 mm in diameter) from PDA cultures to Petri dishes containing PDA and incubating them for 8 days at 28°C. Then they were collected by pipetting 5 ml of sterile water and gently rubbing the surface of the colonies. Conidia were counted with a hemacytometer and concentration of conidia in suspensions was adjusted to $10^7$ conidia/g of soil.

### Soil

Used soil was collected in the Doukkala region. Its physico-chemical properties were: pH 8.08; total phosphorus 0.0325%; organic carbon 0.85%; total nitrogen 0.37%; electrical conductivity 413 µs/cm.

### Effects of fungicides on sclerotial viability in the soil

Three fungicides were tested: benomyl at 25, 50 and 250 mg a.i./$m^2$, oxyquinoleine at 21, 42 and 210 mg a.i./$m^2$ and hymexazol at 54, 108 and 540 mg a.i./$m^2$.

Plots of 1 $m^2$ of surface area were covered with a layer of soil 15 cm deep. In each plot, the soil was sprayed with one of the fungicide suspensions and samples were taken and placed in plastic bags (25 g of soil per bag). Twenty sclerotia of *S. rolfsii* were added to each bag and the soil was adjusted to 70% of moisture holding capacity. The bags were sealed and incubated at 28°C. A control contained soil without fungicide but with the 20 sclerotia of *S. rolfsii*.

Other soil was sterilized and used to cover three plots of 1 $m^2$ each. Each plot was then treated with one of the fungicides: benomyl at 250 mg a.i./$m^2$, hymexazol at 540 mg a.i./$m^2$ and oxyquinoleine at 210 mg a.i./$m^2$. The treated sterilized soil was placed in plastic bags (25/bag) to each of which 20 sclerotia of *S. rolfsii* were added.

All treatments were replicated six times in each trial.

After four weeks, the sclerotia were removed, disinfected with 1% sodium hypochlorite for 3 min and their viability was determined by incubation on PDA at 28°C for 72 h.

### Effect of *T. harzianum* on sclerotial viability in soil treated with fungicides

The test of the effect of *T. harzianum* isolates on the viability of sclerotia of *S. rolfsii* was realized according to the method of Artigues and Davet (1984). To each plastic bag with 25 g of treated soil plus 20 sclerotia of *S. rolfsii*, was added a conidial suspension of *T. harzianum* at a concentration of $10^7$ conidia/g of soil. The soil was mixed and adjusted to 70% of moisture holding capacity. Each treatment was replicated six times.

After four weeks, the sclerotia were removed from the soil, disinfected with 1% sodium hypochlorite for 3 min and their viability was determined.

### Effect of fungicides on mycelial growth of *T. harzianum* isolates and *S. rolfsii* in agar medium

The fungicides were added to Czapek-Dox agar at three concentrations: benomyl at 5, 10 and 50 µg a.i./ml; oxyquinoleine at 4, 8 and 40 µg a.i./ml and hymexazol at 6, 12 and 60 µg a.i./ml. The control consisted of Czapeck-Dox agar without fungicide. Mycelial disks (7 mm in diameter) were transferred from the margin of 4-day-old colonies of *T. harzianum* isolates or *S. rolfsii* to the center of plates with each fungicide. Six Petri dishes were used for each treatment.

Mycelium growth was assessed by measuring the colony diameter in two perpendicular directions after 48 h at 28°C, and the inhibition percentage was determined.

## Results

### Effect of the fungicides on sclerotia viability

Sclerotia viability was significantly reduced by the three fungicides as compared with the control (Table 1). Benomyl showed the greatest reduction, producing 53.3% of non-viable sclerotia at a concentration of 50 mg a.i./$m^2$ and 68.3% at 250 mg a.i./$m^2$. Hymexazol reduced sclerotia viability less than benomyl, with the proportion of non-viable sclerotia greater than 50% only at the concentration of 540 mg a.i./$m^2$. Oxyquinoleine exhibited the least effect on sclerotia, with a less than 50% reduction in sclerotia viability at all concentrations.

There was no significant difference between natural and sterilized soils in the effectiveness of the three fungicides at the tested concentrations (Table 1).

### Effect of fungicides and *T. harzianum* on sclerotia viability

The four isolates of *T. harzianum* significantly reduced the viability of sclerotia compared with the control (Table 1). This antagonistic effect of *T. harzianum* differed however depending on the fungicide added. Hymexazol enhanced the antagonistic ability of all *T. harzianum* isolates and the proportion of non-viable sclerotia increased significantly with increasing rate of hymexazol, being greatest at 108 and 540 mg a.i./$m^2$. With 108 mg/$m^2$ of

Table 1. Effect of fungicide plus one of four *Trichoderma harzianum* isolates (ES, KB2, NZ, KF1) on sclerotia viability in soil after four weeks of incubation at 28°C.

| Fungicide | | Percentage of non-viable sclerotia | | | | |
|---|---|---|---|---|---|---|
| Type | Concentration mg a.i./$m^2$ | without *T. harzianum* | with *T. harzianum* | | | |
| | | | ES | KB2 | NZ | KF1 |
| Natural soil | | | | | | |
| Untreated control | 0 | 5.8 e | 53.3 de | 70.8 c | 65 c | 49.2 de |
| Benomyl | 25 | 35.8 cd | 48.3 e | 50 d | 46.7 de | 40.0 de |
| | 50 | 53.3 ab | 49.2 e | 51.7 d | 54.2 cd | 33.4 e |
| | 250 | 68.3 a | 63.3 cde | 75 c | 70 c | 58 cd |
| Oxyquinoleine | 21 | 5.0 e | 60.0 de | 50.8 d | 46.0 de | 65.7 cd |
| | 42 | 7.5 e | 70.0 cde | 32.5 e | 33.3 e | 72.5 bc |
| | 210 | 46.7 bc | 76.7 c | 51.7 d | 61.7 cd | 81.7 b |
| Hymexazol | 54 | 19.2 d | 67.5 cd | 90.0 b | 60.8 c | 58.3 cd |
| | 108 | 28.3 cd | 85.8 b | 95.0 a | 83.3 b | 84.2 b |
| | 540 | 57.5 ab | 100 a | 98.0 a | 100 a | 99.2 a |
| Sterilized soil | | | | | | |
| Benomyl | 250 | 67.5 a | - | - | - | - |
| Oxyquinoleine | 210 | 50.8 bc | - | - | - | - |
| Hymexazol | 540 | 60.0 ab | - | - | - | - |

Means in each column followed by the same letters are not different statistically ($P$=0.05) according to Newman and Keuls' test.
-, not determined.

hymexazol, the percentages of non-viable sclerotia for NZ and KB2 isolates were 83 and 95% respectively.

At 25 and 50 mg a.i./$m^2$ benomyl reduced the antagonistic effect of all *T. harzianum* isolates, with fewer non-viable sclerotia in the soil as compared with untreated soil; when it was applied at 250 a.i./$m^2$ on the other hand, the number of non-viable sclerotia was slightly greater for *T. harzianum* isolates ES and KF1, while it remained statistically the same for KB2 and NZ.

The antagonistic ability of *T. harzianum* was influenced by oxyquinoleine depending on the isolates. The antagonistic effect of KF1 and ES isolates was slightly improved by the fungicide, while with NZ and KB2 was weakened compared to the control (Table 1).

**Effect of fungicide in agar medium**

*T. harzianum* isolates differed in their response to the tested fungicides (Table 2). The mycelial growth of all *T. harzianum* isolates was reduced by benomyl and oxyquinoleine, with the highest fungicide concentration causing the greatest reduction in mycelial growth. At the highest concentrations, mycelial growth was completely inhibited. The effect of benomyl was stronger than that of oxyquinoleine. With benomyl, the percentage of inihibition was higher than 50% at the lowest concentration (5 µg a.i./ml ) for all isolates of *T. harzianum* (Table 2). By contrast, hymexazol caused only slight inhibition of the growth of KB2, NZ and KF1 isolates of *T. harzianum*.

*S. rolfsii* growth was reduced by all tested fungicides, but to varying extent. Hymexazol reduced growth more than benomyl and oxyquinoleine at all concentrations (Table 2 ). At the two lowest concentrations, the inhibition caused by benomyl and oxyquinoleine was the same. However, at the highest concentration, the inhibitory effect of benomyl was greater than that of oxyquinoleine.

## Discussion

The effect of fungicides on the viability of sclerotia of *S. rolfsii* was similar in natural and sterilized soils. This may indicate that interaction between the tested fungicides and the indigenous soil mycoflora was negligible. Any reduction of sclerotia viability in treated natural soil may therefore be attributed to the fungicide directly.

The response of the *T. harzianum* isolates and *S. rolfsii* to fungicides in culture medium may explain the different interactions observed in the soil. Thus, benomyl strongly inhibited the different *T. harzianum* isolates reducing their antagonistic capacity, notably at low concentrations (25 and 50

Table 2. Effect of fungicides on the mycelial growth of *Trichoderma harzianum* isolates (ES, KB2, NZ, KF1) and *Sclerotium rolfsii* on PDA incubated at 28°C for 48 h.

| Fungicide | | Percentage of inhibition of mycelial growth | | | | |
|---|---|---|---|---|---|---|
| Type | Concentration µg a.i./ml | ES | KB2 | NZ | KF1 | *S. rolfsii* |
| Benomyl | 5 | 64 c | 62 c | 72 b | 64 c | 17 d |
| | 10 | 72 b | 71 b | 74 b | 72 b | 15 d |
| | 50 | 100 a | 100 a | 100 a | 100 a | 43 b |
| Oxyquinoleine | 4 | 8 e | 21 e | 16 d | 25 e | 11 d |
| | 8 | 26 d | 43 d | 41 c | 30 d | 10 d |
| | 40 | 100 a | 100 a | 100 a | 100 a | 28 c |
| Hymexazol | 6 | 0 e | 8 g | 0 e | 2 h | 33 c |
| | 12 | 0 e | 15 f | 0 e | 5 g | 43 b |
| | 60 | 0 e | 12 f | 18 d | 13 f | 70 a |

Means in each column followed by the same letter are not different statistically ($P$=0.05) according to Newman and Keuls' test.

mg a.i./$m^2$). The increase in non- viable sclerotia at the highest tested fungicide concentration (250 mg a.i./$m^2$) may be a direct effect of the fungicides. This result corroborate previous findings that benomyl and other benzimidazoles (thiabendazol, thiophanate-methyl) are effective inhibitors of *T. harzianum* (Davet, 1979; Papavizas, 1985).

Oxyquinoleine on the whole inhibited *T. harzianum*, but the effect depended strongly on the isolate. As a result soil treated with this fungicide could either slightly improve or weaken the antagonistic action of *T. harzianum*.

By contrast, hymexazol showed poor activity against *T. harzianum* in agar medium even at the highest concentration tested (60 µg a.i./ml). This fungicide therefore enhanced the antagonism of *T. harzianum* isolates. This greater antagonism can be explained in two ways: as due to a change in the balance of the mycoflora of the soil in favor of *T. harzianum*, as was suggested by Elad *et al.* (1980) and Davet and Martin (1985), or to an enhanced penetration capacity of *Trichoderma* isolates into sclerotia as reported by Henis and Papavizas (1983).

Hymexazol, a fungicide of the isoxasol family, is recommended mainly against soilborne fungal pathogens belonging to the genus *Pythium*, *Aphanomyces*, *Corticium* and *Fusarium* (Cluzeau, 1997). As a result, we found that this fungicide had no effect against *T. harzianum*, nor had other fungicides such as PCNB, captan, chlorothalonil, iprodione, vinchlozoline or metalaxyl (Papavizas, 1985). Some of these fungicides increased the effectiveness of *T. harzianum* as a sclerotial control agent (Davet and Martin, 1985; Mukherjee *et al.*,1989). In the case of *S. rolfsii* diseases, a inhibitoty effect was reported by the simultaneous application of either PCNB plus *T. harzianum* (Chet *et al.*, 1979) or methyl bromide plus *T. harzianum* (Elad *et al.*, 1980).

Hymexazol has only a limited effect on sclerotia viability. However, a combination of hymexazol with any of the tested isolates of *T. harzianum* showed a good synergistic interaction even at a low concentration of 108 mg a.i./$m^2$, which is 20% of the usual rate.

On the other hand, Henis (1984) on the effectiveness of *T. harzianum* isolates against *S. rolfsii* in soil plates reported that the effect on sclerotia was the best criterion for biological control capacity. It is therefore concluded that the use of hymexazol in combination with the tested isolates of *T. harzianum* against *S. rolfsii* is a promising approach in integrated disease control.

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