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Original article

Effect of nitrogen fertilizers and *Trichoderma harzianum* on *Sclerotium rolfsii*

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Abstract – The effect of urea, sulfate ammonium, nitrate potassium and horse manure on *S. rolfsii* was tested in vitro, alone and in combination with *T. harzianum*. Tests on liquid culture media showed that *Sclerotium rolfsii* did not utilize urea as a source of nitrogen while sulfate ammonium and nitrate potassium allowed the growth of the fungus. On a solid medium, the fertilizers at rates of 12 g $N \cdot m^{-2}$ and 18 g $N \cdot m^{-2}$ had an inhibitory effect on the growth of *Sclerotium rolfsii* mycelium. The highest effect was observed for urea. In contrast, *Trichoderma harzianum* assimilated all fertilizers but had a preference for sulfate ammonium. The antagonistic activity of *Trichoderma harzianum* on *Sclerotium rolfsii* on solid culture media was stimulated in the presence of the three nitrogen sources. The horse manure at high rates inhibited the growth of *S. rolfsii,* favored the development of *T. harzianum* and enhanced its antagonistic effect on *S. rolfsii.* The confrontation of *Trichoderma harzianum* with sclerotia of *Sclerotium rolfsii* in soil fertilized separately with urea, sulfate ammonium, nitrate potassium or manure showed an increase in the antagonistic activity. Particularly, the manure in combination with *T. harzianum* induced high mortality of sclerotia of the fungus. In the agronomic context of the region of Doukkala, it seems adequate to add these nitrogen sources to contribute to the biological control of *Sclerotium rolfsii*.

antagonism / *Trichoderma harzianum* / *Sclerotium rolfsii* / fertilizers / manure

1. INTRODUCTION

Sclerotium rolfsii is a widely distributed pathogen of many crops in the tropical and Mediterranean regions. In Morocco, it is economically significant on sugar beet in the irrigated area of Doukkala. It causes a root rot that appears late in the season. The disease is very damaging to the crop when harvested in late July or early August. The losses can be up to 50% of the root production in some situations [12, 13].

There is no economically effective chemical control against *Sclerotium* root rot on sugar beet. Preventive methods such as some cultural practices [early harvesting and crop rotation] and soil solarization have been proposed as means of control in many studies [14, 19, 29, 36, 39]. Nitrogen fertilizers and organic amendments were found to reduce the incidence of the disease in various experiments [18, 19, 26, 34, 40]. Biological control of *S. rolfsii* is an alternative that offers possibilities of reducing the effect of this pathogen on crops. The antagonistic activity of fungi such as *Trichoderma* species on *S. rolfsii* has been demonstrated in many studies [1, 2, 8, 16]. *Trichoderma* species were also found to reduce the incidence of diseases caused by *S. rolfsii* under controlled conditions [6, 16, 27]. However, the efficacy of the antagonistic activity of *Trichoderma* species under natural conditions depends largely on the physical, chemical and biological conditions of the soil [5, 7, 8, 21].

In order to enhance the antagonistic activity of *Trichoderma*, some authors have evaluated the combined effects of the antagonists with practices such as soil fumigation, solarization and fungicide application [7, 10, 23]. However, few studies have been devoted to evaluating the combined effects of *Trichoderma* and nitrogen fertilizers. Matti and Sen [27] reported the synergic action of some fertilizers and *Trichoderma* on *S. rolfsii*. More recently, Bulluck and Ristaino [4] found that organic amendments reduced the incidence of the disease caused by *S. rolfsii* and favored the proliferation of the antagonistic micro-flora of the soil, especially *Trichoderma* species.

In the irrigated area of Doukkala, the intensive sugar beet cropping is characterized by heavy inputs of nitrogen fertilizers [38]. This intensive cropping tends to reduce the fertility and the organic content of the soils [38]. Thus, in the search for a biological method to control *S. rolfsii*, it is important to know the effect of nitrogen fertilizers on the development of the pathogen and on the activity of antagonists in the soil. For this purpose, the present work is aimed at studying the effect of three mineral amendments and one organic manure on the development of *S. rolfsii* and on the antagonistic activity of *T. harzianum*.

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2. MATERIALS AND METHODS

2.1. Fungal isolates

The isolate Kb_2 of *T. harzianum* used was collected from the Doukkala region. The inoculum of *Trichoderma* was prepared on a mixture of: 200 g of sand, 6 g of oatmeal and 30 ml of sterile distilled water. The substrate was homogenized, placed in 250 ml flasks and autoclaved. Each flask was inoculated by five mycelial disks (5 mm in diameter) of *T. harzianum.* Cultures were incubated at 30 °C. The isolate of *S. rolfsii* was originally derived from infested tuber of sugar beet taken from the Doukkala region.

Production of sclerotia was prepared on a mixture of sand and wheat bran [2/3, 1/3 V/V]. The substrate was homogenized and placed in 250 ml flasks, humidified with distilled water and autoclaved. In each flask, we added 50 ml of sterilized water agar at 1%. Five mycelial disks (5 mm in diameter) of *S. rolfsii* were transferred to each flask. Cultures were incubated at 30 °C in the dark for 5 weeks. The formed sclerotia were harvested, dried and stored at 4 °C until further use.

2.2. Soil

The soil used was collected from the Doukkala region. Its physico-chemical properties were: pH 8.08, organic carbon 0.85%, total nitrogen 0.37% and electrical conductivity 413 $\mu s \cdot cm^{-1}$.

2.3. Amendments

The fertilizers used were: urea 46%, sulfate ammonium 21% and nitrate potassium (13% of nitrogen and 44% of oxyde potassium). They were used at 6, 12 and 18 g $N \cdot m^{-2}$, rates that corresponded to 60, 120 and 180 kg $N \cdot ha^{-1}$, respectively. The horse manure was used at 2, 4 and 6 $kg \cdot m^{-2}$ that corresponded to 20, 40 and 60 $tons \cdot ha^{-1}$. Its physico-chemical properties were: pH 7.34, organic carbon 9.84, total nitrogen 3.808 $mg \cdot g^{-1}$ and electrical conductivity 4.497 $ms \cdot cm^{-1}$.

2.4. Effect of amendments on sclerotial viability in the soil

Plots of 1 m^2 of surface area were covered with a layer of soil 15 cm deep. In each plot, the soil was treated with one of the amendments 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 30 °C. A control contained soil without fertilizer but with the 20 sclerotia of *S. rolfsii*.

Other soil was sterilized (45 min at 120 °C in the autoclave) and used to cover four plots of 1 m^2 each. Each plot was treated with one of the amendments: urea, sulfate ammonium or nitrate potassium at 18 g $N \cdot m^{-2}$ and horse manure at 6 $kg \cdot m^{-2}$. The treated sterilized soil was placed in plastic bags (25 g per 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 potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) at 30 °C for 72 h.

2.5. Effect of *T. harzianum* on sclerotial viability in the amended soil

The antagonistic effect of *T. harzianum* against *S. rolfsii* was realized according to the method of Artigues and Davet [2]. To each plastic bag containing 25 g of the amended soil and 20 sclerotia of *S. rolfsii*, we added a conidial suspension of *T. harzianum* at a concentration of 10^7 $conidia \cdot g^{-1}$ 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 hypo-chlorite for 3 min, and their viability was determined.

2.6. Study of in vitro antagonistic action in the presence of amendments

Samples of the soil treated by urea, sulfate ammonium or nitrate potassium at different rates were distributed in Petri dishes (12 g per dish) and autoclaved at 120 °C for 15 min: to each dish, we added 15 ml of autoclaved Czapeck-Dox agar without nitrogen. In the Petri dishes containing soil treated by manure, we added autoclaved water agar at 2%.

After solidification of the medium-soil, a lot of the Petri dishes containing the treated soil were used to determine the fertilizer or manure effects on the mycelial growth of *T. harzianum* or *S. rolfsii*. The centers of the Petri dishes were inoculated by agar disks (7 mm in diameter) from the margin of 6-day-old colonies of *T. harzianum* or *S. rolfsii.*

The controls were prepared with the non-treated soil. Each treatment was replicated six times. For each treatment, the mycelial growth of *T. harzianum* or *S. rolfsii* was assessed after 3 days of incubation at 30 °C by measuring the colony diameter in two perpendicular directions. The comparison of the amendments' effects on the mycelia growth of *S. rolfsii* or *T. harzianum* was determined by the rate of increase:

$$A\% = (C_a - C_o/C_o) \times 100$$

C_a: linear growth of *S. rolfsii* or *T. harzianum* in Petri dishes containing the amended soil;

C_o: linear growth of *S. rolfsii* or *T. harzianum* in Petri dishes containing the non-treated soil.

The other lot of Petri dishes was used to study the impact of amendments on the antagonistic action of Kb_2 against *S. rolfsii*. Paired isolates were placed simultaneously on opposite sides. *T. harzianum* isolate was placed 5 cm from that of *S. rolfsii*. The control was prepared with the non-amended soil using the same protocol previously described. Each treatment was replicated six times. After 3 and 6 days of incubation at 30 °C, the mycelial growth was simultaneously assessed for *S. rolfsii* and *T. harzianum* by measuring the colony diameter in two perpendicular directions.

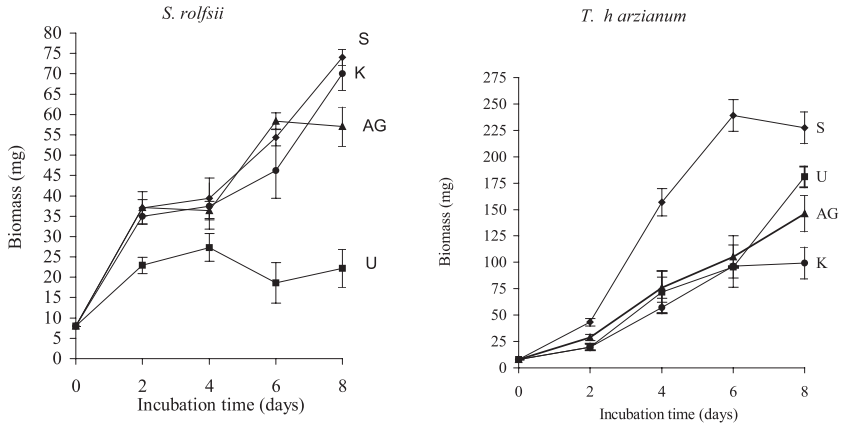

Figure 1. Dry weight (mg) of *S. rolfsii* and *T. harzianum* on Czapeck-Dox medium containing the urea (U), the sulfate ammonium (S), the nitrate potassium (K) or the glutamic acid (AG), measured after 2, 4, 6 and 8 days of incubation at 30 °C.

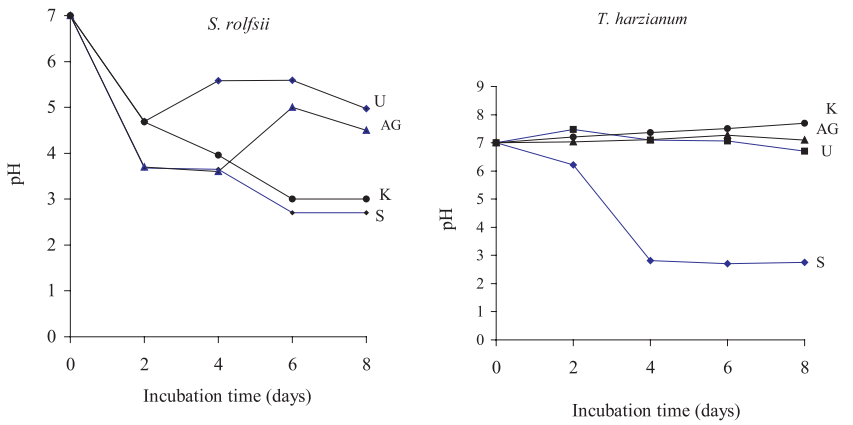

Figure 2. Evolution of the filtrate pH of *S. rolfsii* and *T. harzianum* cultured in Czapeck-Dox medium containing the urea (U), the sulfate ammonium (S), the nitrate potassium (K) or the glutamic acid (AG).

2.7. Effect of different nitrogen fertilizers on the biomass of *T. harzianum* isolate and *S. rolfsii*

The medium used in this experiment was the Czapek-Dox to which various nitrogen compounds: urea, sulfate ammonium, nitrate potassium or glutamic acid were added as the source of nitrogen to get C/N = 20. The pH was adjusted to 7 and the medium was distributed in Erlenmeyers of 250 ml capacity and autoclaved at 120 °C for 15 min. Each Erlenmeyer, containing 100 ml of medium, was inoculated by mycelial disks (7 mm in diameter) that were transferred from the margin of 7-day-old colonies of *T. harzianum* isolate (Kb_2) or *S. rolfsii*. Each treatment was replicated four times. The cultures were placed on a shaking table and incubated at 30 °C. They were filtered 2, 4, 6 and 8 days after incubation. The mycelium was dried for 24 hours at 60 °C and the weight was determined. Simultaneously, the pH of the culture filtrates was measured for each sample.

3. RESULTS AND DISCUSSION

3.1. Effect of nitrogen fertilizers on the development of *S. rolfsii*

Sulfate ammonium, nitrate potassium and glutamic acid allowed mycelial growth of *S. rolfsii* in a liquid medium. This growth was similar for the three nitrogen sources, except the small diminution observed in the case of glutamic acid after the 8th day of incubation (Fig. 1). In contrast, in the medium containing urea, the fungus expressed a poor growth that became constant after the 4th day of incubation (Fig. 1). The biomass produced at the 8th day of incubation was 74, 70 and 57 mg for sulfate ammonium, nitrate potassium and glutamic acid, respectively, while it was only 22 mg in the medium containing urea. In parallel, the pH of the culture filtrates decreased significantly when the growth of *S. rolfsii* was significant (Fig. 2). This was the case of the filtrates from the cultures incubated in the medium containing the sulfate ammonium or the nitrate potassium at the 6th day of incubation. The pH was 2.7 and 3, respectively, whereas in the filtrates of cultures incubated in a medium containing the urea the pH remained near 6. This result showed that both sulfate ammonium and nitrate potassium were better used by *S. rolfsii* as a nitrogen source than glutamic acid and urea, that was obviously badly used. The acidification of the media in which the growth of *S. rolfsii* was significant can be explained by the liberation of oxalic acid by the growing fungus [3].

3.2. Effect of nitrogen fertilizer rate on the mycelial growth of *S. rolfsii*

Czapeck-Dox agar medium, containing the fertilized soil, the growth of *S. rolfsii* was improved with sulfate ammonium

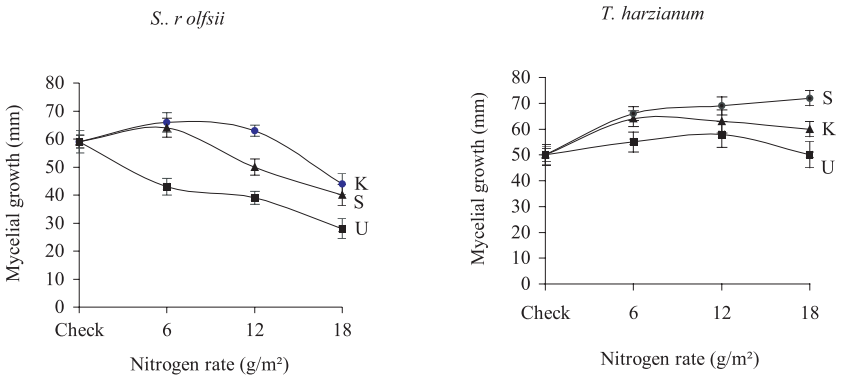

Figure 3. Mycelial growth of *T. harzianum* and *S. rolfsii* after 72 hours of incubation on a medium constituting CzapeckDox agar and soil amended with sulfate ammonium (S), nitrate potassium (K) or urea (U) at the rates of 6, 12 and 18 g of nitrogen·m^{-2}.
Check: Czapeck-Dox agar and soil without fertilizer.

or nitrate potassium at the rate of 6 g N·m^{-2}. The radial growth of *S. rolfsii* was of 66 mm and of 64 mm at 6 g·m^{-2} of nitrate potassium and sulfate ammonium, respectively, while it was 55 mm in the check. However, the growth of the fungus slowed down at high rates of these fertilizers (Fig. 3). The urea had a clear negative effect on the growth of *S. rolfsii*. The radial growth of the fungus was less significant than that of the check even at the feeble rate (6 g N·m^{-2}) and decreased notably at the high rates. It was 43, 39 and 28 mm for 6 g N·m^{-2}, 12 g N·m^{-2} and 18 g N·m^{-2} of this fertilizer (Fig. 3).

The sulfate ammonium and the nitrate potassium, although utilized as a source of nitrogen by *S. rolfsii*, have an inhibitory effect on the fungus at the high rates, 12 g N·m^{-2} and 18 g N·m^{-2}, which are equal to 120 kg N·ha^{1} and 180 kg N·ha^{1}. Nitrogen fertilizers were reported to reduce disease caused by *S. rolfsii* under field conditions by many authors [18, 19, 26, 34, 40]. The direct effect of nitrogen fertilizers on the growth of *S. rolfsii* noted in our study supports these findings. However, our results show that for the same rate of nitrogen, the inhibitory effect on *S. rolfsii* varied according to the type of fertilizer. Thus, among the three fertilizers tested, the urea expressed the most inhibited effect of the fungus. This fertilizer exhibited an adverse effect even at the low rate, 6 g·m^{-2}, that is equal to 60 kg N·ha^{-1}.

3.3. Effect of nitrogen fertilizers on the development of *T. harzianum*

The sulfate ammonium allowed the most significant growth of *T. harzianum* in the liquid medium, compared with the other sources of nitrogen (Fig. 1). In fact, the biomass of *T. harzianum* obtained after 8 days of incubation was 227.6 mg in the case of sulfate ammonium while this bio-mass was 180.9, 146 and 99.3 mg in the case of urea, glutamic acid and nitrate potassium, respectively (Fig. 1). The pH of the cultures' filtrate from the media containing the last three fertilizers changed little. It varied around 7 (Fig. 2). However, in the case of the sulfate ammonium, we noted an acidification of the culture filtrate. After 4 days of incubation the pH decreased from 7 to 2.75.

3.4. Effect of nitrogen fertilizer rate on the mycelial growth of *T. harzianum*

On the Czapeck-Dox agar medium, containing one of the fertilizers at 6 g N·m^{-2}, the growth of *T. harzianum* was more significant than for the check without any amendment. The most significant growth of *T. harzianum* was obtained in the medium containing sulfate ammonium. This growth increased slowly with the rate of the fertilizer. It went from 66 mm to 72 mm at the rates of 6 g N·m^{-2} and 18 g N·m^{-2}, respectively. Unlike the urea and the nitrate potassium, we noted at high rates a small diminution of the growth of *T. hazianum* (Fig. 3).

The three fertilizers were utilized differently by *T. harzianum*. The nitrate potassium and the urea were relatively utilized by *T. harzianum*. However, these fertilizers seemed to have an adverse effect on the fungus at high rates like 18 g N·m^{-2}, which is equal to 180 kg N·ha^{-1}.

The sulfate ammonium was the most easily assimilated by this fungus compared with the other two fertilizers. The *Trichoderma* species were found to have a preference of the ammonium ion [32, 41]. However, this fertilizer may have also indirectly improved the growth of *T. harzianum* by the acidification of the medium. In fact, it has been reported by many authors that an acid pH was favorable to the development of *Trichoderma* and its antagonistic ability [5, 21, 24]. In practice, the repeated application of sulfate ammonium acidifies the soil [15]. The use of this fertilizer could therefore be advantageous in the case of Doukkala soils, which are alkaline.

3.5. Effect of nitrogen fertilizers on the antagonistic action of *T. harzianum* against the mycelial growth of *S. rolfsii*

Dual cultures of *T. harzianum* and *S. rolfsii* on the Czapeck-Dox agar medium, containing one of the fertilizers, showed an increase in the inhibitory effect of *T. harzianum* on the mycelial growth of *S. rolfsii* at all tested rates (Fig. 4). For instance, after 72 hours of incubation, the linear growth of *S. rolfsii* was 25, 21 and 15 mm at the rate of 6 g N·m^{-2} of sulfate ammonium, urea and nitrate potassium, respectively, while it was 51 mm for the check.

The comparison of the effect of the three fertilizers revealed that nitrate potassium has the most significant effect on the inhibitory action of *T. harzianum* against *S. rolfsii*. In addition, qualitative variations were recorded in the antagonistic activity depending on the type of fertilizer: (i) in the case of nitrate potassium, we noted a browning of the mycelium in the zone of the contact between *S. rolfsii* and *T. harzianum* corresponding to dead mycelium. This zone has a wide variation between

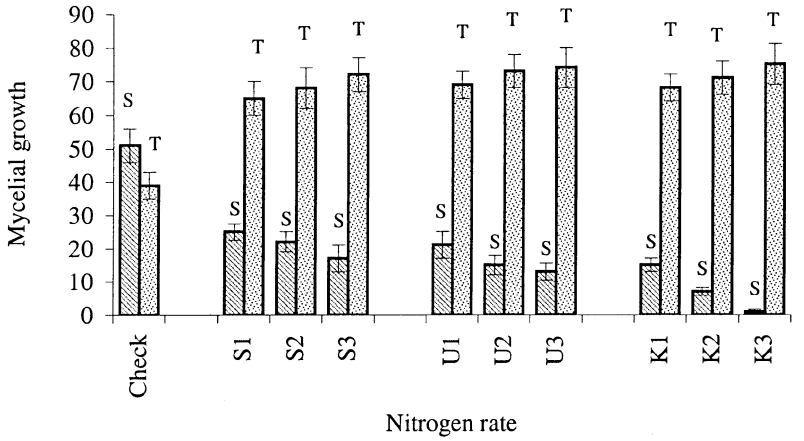

Figure 4. Mycelial growth of *T. harzianum* (T) and *S. rolfsii* (S) after 72 hours of incubation in dual culture on the medium containing Czapeck-Dox and soil amended with the sulfate ammonium (S), the nitrate potassium (K) or the urea (U) at the rates of 6 (S1, U1, K1), 12 (S2, U2, K2) and 18 g of nitrogen·m^{-2} (S3, U3, K3).

2 and 10 mm according to the rate. Microscopic examination of the hyphal fragment taken from this zone revealed that *T. harzianum* mycelia coiled around that of the pathogen; (ii) in the case of the urea, the formed mycelium of *S. rolfsii* in dual culture was particularly sparse, and (iii) for the sulfate ammonium, the confrontation between *T. harzianum* and *S. rolfsii* was marked by the presence of an inhibitory zone of 5 to 8 mm wide.

The three nitrogen fertilizers expressed a stimulatory effect on the antagonistic activity of *T. harzianum* at all rates. In fact, the antagonistic action was weak in the check cultures growing on a medium poor in nitrogen. Urea and nitrate potassium, even though they are not favorable to the growth of *T. harzianum*, seemed to activate clearly the antagonistic activity of this fungus against *S. rolfsii*.

The dual culture trial of *T. harzianum* and *S. rolfsii* on a culture medium containing one of the fertilizers revealed qualitative differences in the expression of the antagonistic action in relation to the type of fertilizer. The mechanisms of antagonism of *Trichoderma* are complex and diversified, such as antibiosis and mycoparasitism [17]. Many compounds released by *Trichoderma* were implicated in these mechanisms such as toxin and cell wall enzyme degradation [17, 30] It seems therefore that each fertilizer stimulates relatively one of the mechanisms involved in the antagonistic action of *Trichoderma,* particularly the type of enzyme and toxin production. In fact, it has been reported that the nitrogen sources, such as sulfate ammonium, nitrate potassium and urea, influence specific enzyme production by *Trichoderma* [9, 11, 22].

In conclusion, the nitrogen fertilizers have, even at low rates, a great stimulatory effect on the antagonistic activity of *T. harzianum* and seem to influence directly or indirectly the mechanisms involved in this activity.

3.6. Effect of manure on the development of *S. rolfsii*

The mycelial growth of *S. rolfsii* was improved significantly on the medium, containing water agar and soil at a low rate of manure, 2 kg·m^{-2}. This tendency is reversed when the rate of manure is doubled or tripled (Fig. 5). Thus, after three days of incubation, the radial growth of *S. rolfsii* after 72 hours of

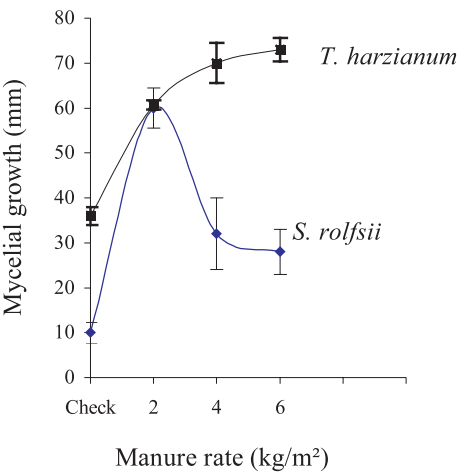

Figure 5. Mycelial growth of *T. harzianum* and *S. rolfsii* after 72 hours of incubation on a medium constituting water agar and soil amended with the horse manure at the rates of 2, 4 and 6 kg/m^2. Check: water agar and soil without horse manure.

incubation was 60, 32 and 28 mm at the manure rates of 2, 4 and 6 kg·m^{-2}, respectively.

S. rolfsii utilized the horse manure as a source of carbon and of nitrogen. The growth of the fungus was improved by a low rate of the manure. However, the reduction of the fungal growth at high rates can be explained by the presence in the manure of inhibitory compounds that directly affect *S. rolfsii*. Organic compounds, and industrial and agricultural composts, have variable effects on *S. rolfsii*. Some of these compounds were reported to favor the development of *S. rolfsii* [4, 20, 33]. Other amendments, such as swine manure, cotton–gin trash and ground mesocarp fiber of oil palm have negative effects on *S. rolfsii* [4, 33]. The horse dung that we tested has an inhibitory effect on the growth of *S. rolfsii* at the rates of 4 and 6 kg·m^{-2}, which are equal to 40 and 60 tons·ha^{-1}. The effect of this compound on the fungus and on the incidence of the disease it causes needs to be tested in the field.

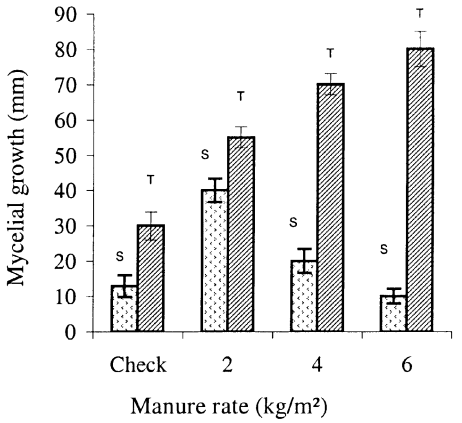

Figure 6. Mycelial growth of *T. harzianum* (T) and *S. rolfsii* (S) after 72 hours of incubation in dual culture on a medium constituting water agar and soil amended with the horse manure at the rates of 2, 4 and 6 kg/m^2.

3.7. Effect of manure on *T. harzianum* and on its antagonistic action against *S. rolfsii*

The radial growth of *T. harzianum* was improved on medium containing water agar and soil amended with the horse manure. This growth was of 60, 70 and 73 mm at the manure rates of 2, 4 and 6 kg·m^{-2}, respectively, compared with 36 mm in the check (Fig. 5). The dual culture of *T. harzianum* and *S. rolfsii* on the medium, containing soil amended with the horse manure, resulted in an inhibitory effect of the mycelial growth of *S. rolfsii,* which was exhibited clearly at a high rate (Fig. 6). Thus, at a low rate (2 kg·m^{-2}), the growth of *S. rolfsii* was not effectively inhibited by *T. harzianum*. The mycelia of the fungi intercrossed mutually. However, at 4 and 6 kg·m^{-2} of manure, the mycelium of *T. harzianum* overcame partially or completely the colony of *S. rolfsii*. Therefore, it seems that the manure provided a food base for the development of *T. harzianum* and may also have stimulated its antagonistic activity.

3.8. Effects of nitrogen fertilizers on the viability of sclerotia of *S. rolfsii*

In the soils containing the sulfate ammonium, the nitrate potassium or the urea at the low rate, 6 g N·m^{-2}, the proportion of sclerotial mortality was significantly small compared with the check without any fertilizer (Tab. I). However, with the high rate of the added fertilizer, the proportion of dead sclerotia was much more significant. It was 27.5, 39 and 41% for 18 g N·m^{-2} of urea, sulfate ammonium and nitrate potassium, respectively, while it was 6% in the check.

Few studies have been interested in the effect of nitrogen fertilizers on sclerotia of *S. rolfsii*. Matti and Sen [27] have reported that calcium ammonium applied at 40 kg N·ha^{-1} had no effect on the viability of sclerotia while urea at the same rate significantly reduced viable sclerotia. Also, Hoynes [18] has found that sulfate ammonium, nitrate ammonium, diammonium phosphate or urea, applied to soil at a field rate of 135 kg N·ha^{-1} did not reduce the viability of sclerotia of *S. rolfsii*. It seems according to our results that the effect of nitrogen fertilizers on the viability of sclerotia was exhibited at a high rate. Also, the mortality of the sclerotia was significant at the high rate tested in sterilized as well as in natural soil, supporting the evidence of the direct effect of the fertilizers on the sclerotia.

Table I. Combined action of nitrogen fertilizers and *T. harzianum* on the sclerotial viability of *S. rolfsii* in soil.
Means in each column followed by the same letters are not different statistically (P = 0.05) according to Newman & Keuls' test.

AMENDMENT	Rate (kg·ha^{-1})	PERCENTAGE OF DEAD SCLEROTIA	
		Without T. harzianum	*With T. harzianum*
CONTROL	0	6 c	71d
FERTILIZER			
natural soil			
Urea	60	10 c	72.5 cd
Sulfate ammonium		13 bc	76 c
Nitrate potassium		14 bc	88 bc
Urea	120	17.5 bc	78 cd
Sulfate ammonium		32 b	83 c
Nitrate potassium		27 b	95 b
Urea	180	27.5 b	89 bc
Sulfate ammonium		39 ab	92 b
Nitrate potassium		41 a	97 b
sterilized soil	Rate (kg·ha^{-1})		
Urea	180	21 b	
Sulfate ammonium		27.5 b	
Nitrate potassium		35 ab	
MANURE	Rate (t·ha^{-1})		
natural soil			
	20	7 c	87 bc
	40	29 b	97 b
	60	32.5 ab	100 a
sterilized soil	60	21.65 b	

3.9. Combined effects of the nitrogen fertilizers and *T. harzianum* on the viability of sclerotia of *S. rolfsii*

The application of *T. harzianum* in combination with fertilization at a low rate significantly reduced sclerotial viability of *S. rolfsii* compared with the check. Also, the percentage of

sclerotial mortality increased significantly, especially at high rates of nitrogen (Tab. I). Thus, for the rate of 18 g $N \cdot m^{-2}$, the proportion of dead sclerotia was 89, 92 and 97% for the urea, the sulfate ammonium and the nitrate potassium, respectively. This proportion was 71% for the check (Tab. I).

The combined use of *T. harzianum* and the nitrogen fertilizers enhanced their effect on the sclerotia. In our study, this effect was exhibited at a low rate (6 g $\cdot m^{-2}$). Matti and Sen [27] have noted additive effects for the association of *Trichoderma* and urea at 40 kg $N \cdot ha^{-1}$. At this rate, calcium ammonium showed no change in the sclerotial viability. The use of *Gliocladium* in combination with sulfate ammonium or phosphate ammonium at 135 kg $N \cdot ha^{-1}$ was also effective at reducing the viability of sclerotia [18]. Our results indicated that *Trichoderma* and sulfate ammonium, urea or nitrate potassium interfered positively against sclerotia of *S. rolfsii*. These nitrogen fertilizers directly affect sclerotial viability and may be enhanced directly or indirectly by the antagonistic action of *Trichoderma*.

3.10. Effects of manure on the sclerotia of *S. rolfsii* and on the antagonistic activity of *T. harzianum* in the soil

The mortality of sclerotia increased significantly in natural soil amended with the horse dung, especially at the rates 4 and 6 kg $\cdot m^{-2}$ (Tab. I). Similar effects were obtained in sterilized soil. When combined with *T. harzianum*, the added manure had a significant effect on the mortality of the sclerotia. Thus, the proportion of dead sclerotia was 87, 97 and 100% at the manure rates of 2, 4 and 6 kg $\cdot m^{-2}$, respectively. The effect of the manure on the sclerotia in a natural soil can be explained by some actions of the soil micro-flora. In fact, many types of manure have been reported to harbor antagonistic fungi and bacteria [31]. Bulluck and Ristaino (2002) also reported the reduction of the viability of sclerotia in situations where the compost or the manure was added to natural soils.

The reduction of the sclerotial viability, obtained in our study in sterilized soil, supports the evidence of the direct effect of the horse manure on the sclerotia of *S. rolfsii*. In another study, Ramirez et al. [35] reported that the humus significantly affected the viability of sclerotia in a natural soil. The nitrogen compounds resulting from the mineralization process may also have an effect on the viability of sclerotia. Thus, at a high rate, the manure may have directly affected the sclerotial viability of *S. rolfsii.*

The combined use of *T. harzianum* and manure has synergetic action that affected the sclerotial viability. The added manure provided the carbon and the nitrogen to *T. harzianum*. Thus, it enhanced its development and its antagonistic activity. It was reported that soil organic amendments increased the multiplication capacity of *Trichoderma* [25, 37]. Similarly, Bulluck and Ristaino (2002) found that some organic amendments, such as swine manure, increased the population of *Trichoderma* in comparison with some mineral nitrogen fertilizers. Horse manure applied alone or combined with *T. hazianum* has a negative effect on *S. rolfsii.* Soil amendment with horse dung offers an option of control of *S. rolfsii* on sugar beet in the Doukkala area, the soils of which are poor in organic content [38].

4. CONCLUSION

Among the three nitrogen fertilizers, the urea had the most significant effect on the development of *S. rolfsii*. It was poorly utilized as a source of nitrogen by the fungus. This fertilizer also exhibited the greatest inhibitory effect on mycelial growth of *S. rolfsii*. Unlike urea, sulfate ammonium and nitrate potassium were used as a nitrogen source by *S. rolfsii*. They expressed an inhibitory effect on the fungus at a high rate.

The development of *T. harzianum* was enhanced in the presence of one of the three nitrogen fertilizers. Urea and nitrate potassium did not seem to be beneficial to the fungus at a high rate. The sulfate ammonium was the most effective compound at increasing the biomass of *T. harzianum* and enhancing its mycelial growth. The effectiveness of the sulfate ammonium can also be explained by its acidification capacity towards the medium. The acidifying effect of the sulfate ammonium needs to be tested in the soils of the Doukkala area.

The three fertilizers, even at a low rate, 6 g $N \cdot m^{-2}$ (60 kg $N \cdot ha^{-1}$), stimulated the antagonistic effect of *T. harzianum* on the mycelial growth and on the viability of the sclerotia of *S. rolfsii*. The dual culture test of *T. harzianum* and *S. rolfsii* in the presence of one of the fertilizers revealed qualitative variations in the antagonistic activity. These fertilizers may stimulate directly or indirectly the mechanisms involved in the antagonistic action.

The manure inhibited the mycelial growth of *S. rolfsii* and affected the viability of its sclerotia. However, the inhibitory effect on the mycelial growth was expressed only at rates higher than 2 kg $\cdot m^{-2}$. In addition, the manure enhanced growth of *T. harzianum* and its antagonistic effects on the mycelial growth and on the viability of the sclerotia of *S. rolfsii*. Compared with the mineral nitrogen fertilizers, the manure interacts more with *T. harzianum*. Therefore, this compound offers a valuable option of control of *S. rolfsii* in the soils of Doukkala that are poor in organic matter content.

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