Control of fungi diseases on turfgrass using *Trichoderma harzianum*

GUERRERO, C.; VITORIANO, J.; NETO L. AND DIONÍSIO, L.
Faculdade de Engenharia de Recursos Naturais
Universidade do Algarve
Campus de Gambelas, Edifício 8, 8005-139 FARO
PORTUGAL
E-mail: cguerre@ualg.pt

Abstract: - The use of biocontrol agents becomes, nowadays, more and more important for plant disease management acting as a good alternative to the chemical control.
The main objective of the present work was to test the effect of an antagonistic fungus on the control of fungal disease in turfgrass. The experimental work was carried out in nine greens of «Silves Golf», a golf course at southern Portugal. Different concentrations of a commercial product containing *Trichoderma harzianum* were compared to each other.
To isolate pathogenic fungi, samples were taken from all the places where fungal disease symptoms appeared. From these samples, fungi of the genus *Rhizoctonia* (brown patch) and *Lepista* (fairy ring) were isolated. *Fusarium* sp. and *Sclerotinia* sp. were also isolated.
The concentration of *Trichoderma harzianum* concentration in the commercial product was evaluated; a laboratorial trial was carried out to confirm *Trichoderma* presence in the soil + turfgrass due to the commercial product spreading. The identification of pathogenic and antagonistic fungi morphological characteristics were carried out to confirm their presence in the soil + turfgrass samples. *Trichoderma* antagonistic activity was studied.
According to the severity of disease symptoms, differences were observed between plots where the studied product was sprayed and the plots without application (untreated). The disease symptoms appeared, throughout the experimental study, in all plots, but the severity was always higher in the control.
Our results showed that the tested product would be a useful environmental sound approach to control disease of turfgrass in the Algarve region.

Key-Words: - antagonist fungi, biological control, *Fusarium* sp., golf course, *Lepista sp.*, *Rhizoctonia sp.*, *Sclerotinia* sp.

1 Introduction

Golf "industry" is an important activity in the Algarve; the region was voted the "Established Golf Destination of the Year" twice, having previously won the award in 2000, and than in 2006. This title was awarded to the region by the International Association of Golf Tour Operators (IAGTO), gaining preference over such destinations as Andalusia, Arizona, California, Costa Brava, Dubai, France, Valencia, Lisbon and South Africa.

The main reasons for these awards are the high quality standards of Algarve golf courses, the high number of golf courses (the region has nearly half of the total of golf courses open in Portugal), the relatively short distance from each golf course, the accommodations facilities, and the exceptional weather for its practice. These factors are responsible for the high number of people playing this sport in the region, mainly tourists, having each golf course nearly and some times more than 50,000 players across the year.

With this high number of players, turfgrass stresses are usual and high standards of maintenance are necessary to establish and maintain the high quality standard of the golf courses. Water, fertilizers, pesticides, labor, energy and knowledge are needed for achieve the best agronomical practices.

According to diseases occurrence, the main diseases are those promoted by fungi and the principal fungal diseases on turfgrass in Portugal during spring-summer period are dollar spot (*Sclerotinia homeocarpa*) and brown patch (*Rhizoctonia solani*). However, other problematic fungi diseases can occur, such as fairy ring (*Agrocybe* spp., *Marasmius oreads*e, *Lepiota* spp, *Lepista* sp.) and take-all-patch (*Gaeumannomyces graminis*), among others. These are destructive diseases of both cool and warm season turfgrasses [1].

Dollar spot affects a wide variety of grasses, including Kentucky bluegrass, bermuda, perennial ryegrass, zoysia, tall fescue, and bentgrasses. The disease may occur throughout the growing season, especially when there is low soil moisture and an excess of dew or fog. Dollar spot may occur regardless of management of soil fertility, although damage usually is most severe if there is a deficiency of nitrogen. This makes nitrogen as a well know cultural control method for dollar spot [2].

Dollar spot results in the formation of small, roughly circular, bleached patches in the lawn (Fig. 1). The patches are more numerous in areas where there is poor air circulation or drainage. The fungi which cause dollar spot survive indefinitely in thatch and soil. In the presence of a thin film of moisture on leaves and favorable temperatures, these fungi will begin to grow and infect leaves. The fungus apparently does not infect the roots, although toxins produced by this fungus may affect root formation. Dollar spot can be chemical controlled by many contact-nonsystemic and by systemic fungicides, such as fenarimol, propiconazole, triadimefon and iprodione [3].

Fig. 1. Dollar spot symptoms in *Agrostis stolonifera*

Brown patch typically starts to appear during periods of high temperature and high humidity; it mainly occurs on turfgrass with very close cutting height, however it can occur at high cutting. It can develop rapidly when temperatures are warm (20 to 35 °C) and humid, especially on cool-season grasses (fescue, ryegrass, bluegrass and bentgrass). It can also occur on these grasses during warmer periods of the winter months. Warm-season grasses (St. Augustinegrass, Zoysiagrass, Bermudagrass and Centipedegrass) most commonly are affected by brown patch (also called large patch) during the early spring and late fall [2, 3].

The brown patch pathogens normally attack the leaves and later may spread to the crown and roots. The initial symptoms of this disease are circular shaped patches with a diameter of 2.5 to 12.5 cm, and can develop faster up to 60 cm in diameter and fade to a light brown color (Fig. 2).

The fungi can survive saprophytically in the thatch and the fungicides that can be effective against are fenarimol, flutolanil, ipridione, mancozeb, propiconazole, among others [3].

Fairy rings are normally caused by several species of fungus; these fungi inhabit the root zone forming hymenia in the soil; rings may be formed along the developing hymenia. Normally it forms a distinct outer ring of either dark green or dead brown turfgrass (Fig. 3), depending on the agent, which develops different characteristics [2, 3]. The most common species are *Lepista sordida*, *Marasmius orcades*, *Lycoperdon perlatum* and *Agaricus campestris*. All these fungi survive in the saprophytic material. Bicoral, flutolanil and polyoxin are some fungicides effective to many of these fungi species [3].

Fig. 2. Brown patch symptoms in *Agrostis stolonifera*

Fig. 3. Fairy ring symptoms in *Agrostis stolonifera*

The control of fungal diseases may be overcome with control strategies based on the adoption of appropriate cultural practices that can reduce the attacks of these pathogens. In the case of golf courses, with high agronomic maintenance level, these cultural practices may be not enough and chemical control should be necessary.

The possible effects on the environment regarding chemical applications on golf courses are a public concern. Research on degradation and fate of those chemicals and on development of alternative pest control, such as biological control, are an actual trend. According to [4], the leaching of nutrients and pesticides are usual minimal on golf courses. However, public sense enhance the environmental efforts to decrease pesticides applications using alternative cultural practices, such as the increasing of the plant resistance to abiotic and biotic stresses and the introduction of antagonistic organism to combat turfgrass diseases and insects. The intensive use of pesticides can led to the destruction of the soil microbiota and contributes to the reduction of the efficacy of the antagonistic microorganisms.

Pesticides and fungicides are commonly used in golf courses; additionally, high frequency of their use, high costs of agronomical maintenance, development of fungicides resistances and health risks to human and the environment have stimulated the development of alternative methods for disease and pest control [5].

The use of friendly environmental cultural practices may has potential benefits on environment and economy. [6] refers that disease control is the cultural practice where more money is spent in the golf course management.

The use of composts and other organic amendments for disease suppression has the potential to be beneficial in reducing chemical applications for disease control [5, 7]. Although, these authors refer that compost may not control turfgrass fungi diseases to a level without the use of fungicides. The disease suppression normally occurs through a combination of physiochemical and biological mechanisms. The physiochemical action (or factors) depends on compost nutrient levels, organic matter, moisture and pH. According to [5], the biological factors include microbiological populations in the compost, microbiological competition for nutrients with pathogens, antibiotic production, parasitism and predation, among others.

The use of compost, organic fertilizers, or even sludges may be considered a cultural practice, such as the used of any other fertilizer. This, plus the irrigation and thatch control managements may be used for disease control.

In golf courses the use of composts or organic fertilizers in topdressing operations are a new approach to prevent diseases and as the golf course superintendents normally do multiply topdressing with sand of the greens and tees over a season it would not necessarily introduce additional practices and labor in the management program.

Biological control of pests and diseases is attempted by introducing disease suppressive antagonists or by manipulating the activity of antagonistic organisms present in the soil, using microorganism inoculants and soil organic amendments or on plant parts [1, 5, 7, 8].

The genus *Trichoderma* is a potential biocontrol agent against several phytopathogenic fungi [9, 10, 11, 12]. It is also used as biofertilizer because of its ability to establish mycorriza-like association with plants [10]. However, as a fungus, *Trichoderma* spp. is the cause of green mold, a disorder that affects cultivated mushrooms [13].

Some species of genus *Trichoderma* have been tested throughout the years as agents of biological control, being the species more studied *Trichoderma virens*, *Trichoderma harzianum* and *Trichoderma viride* [14]. Under controlled conditions [15] reports partial control using *Trichoderma harzianum*, however in field conditions, results are still not satisfactory.

Trichoderma harzianum has the capacity to protect the root system of several plants (tomato, soy, cotton, ornamental plants among others) against a great diversity of soil fungi, in particular against *Pythium* spp., *Rhizoctonia* spp., *Fusarium* spp. This fungus is considered as a good antagonist and a genuine fungi parasite that can also be used as a promoter of plant growth [16].

The biological mechanisms used by *Trichoderma harzianum* to control the growth of other fungi have been extensively studied. Production of anti-fungal metabolites, extracellular enzymes systems, mycoparasitism have been considered. Antibiotic effect of Trichoderma isolates was also demonstrated [17].

The antagonistic action starts when the *Trichoderma hyphae* turn round the pathogenic fungi hyphae [11]. This fungus parasitism continues with the production of an enzyme that degrades the hosts' cellular wall, creating spaces through nutrients can be extracted for proper fungus growth [11, 18].

The aim of this experimental work was to evaluate at a field scale, the effect of *Trichoderma harzianum* in the biological control of several fungi diseases, such as *Pythium*, *Rhizoctonia*, *Fusarium*,

Sclerotinia and other fungi in turfgrass of golf courses. A suspension of two different concentrations of *Trichoderma harzianum* was spreaded on the soil + turfgrass and compared to where it was not done any inoculation (control treatment). Where fungi disease symptoms occurred, samples were taken in order to identify the pathogenic fungus.

2 Material and Methods

The experimental work was carried out at the Silves Golf Course, located at the south of Portugal (Algarve) (Fig. 4).

It was evaluated the effect of a commercial product containing *Trichoderma harzianum*, which was sprayed to soil + turfgrass in order to evaluate the inoculating efficacy and the antagonistic capacity of this fungus against turfgrass fungi diseases; experiments and microbiological determinations were also developed at the Laboratory of Microbiology of the University of Algarve.

Fig. 4. Silves Golf Course localization (courtesy of the Golf Course)

2.1 Field experimental work

The field experimental work was carried out, in the golf course, on nine greens randomly chosen. Three treatments were evaluated: one with no application of the antagonist (T0) (control treatment) – 9 blocks (3 greens + 6 halves-greens); and two in which it were inoculated 10 kg.ha^{-1} (T10) and 15 kg.ha^{-1} (T15) (3 blocks each) of the commercial product containing *Trichoderma harzianum*. The commercial product used was RIZODERM (LIDA QUÍMICA), which labeled concentration, was 1x10^{8} ufc/g. The inoculation doses and spreading operations were done according to the manufacturer instructions. At total 15 blocks were evaluated and those were randomly distributed among the nine greens (Table 1).

The individual area of each block was determined and recorded. At the beginning of the experimental work a fungicide (fenarimol) was applied in all greens to "remove" any presence of turfgrass pathogenic fungi. After two weeks, the commercial product containing *Trichoderma harzianum* was sprayed on the specific blocks, in two moments with ½ of the treatment dose in each application, separated by 20 days. Before each application, it was evaluated the presence of pathogenic fungi in soil-turf samples. To identify the pathogenic fungi, samples from selected greens were taken from all the places where fungal disease symptoms appeared.

Table 1. Experimental treatments distribution on the golf course (T0 – untreated blocks); T10 - 10 kg.ha^{-1} of the commercial product; T15 - 15 kg.ha^{-1} of the commercial product)

Treatment	Number of green	Treated area
T0	1	all green
T0	11	all green
T0	16	all green
T0	2	half-green
T0	8	half-green
T0	17	half-green
T0	3	half-green
T0	10	half-green
T0	18	half-green
T10	2	half-green
T10	8	half-green
T10	17	half-green
T15	3	half-green
T15	10	half-green
T15	18	half-green

After the first application of the commercial product it was evaluated the occurrence of disease symptoms. All symptoms of illnesses that occurred in all the study blocks were recorded. From the symptom disease areas samples were collected for fungal identification. The visible disease spots/rings were counted per green (block) and it were measured their diameters and calculated the affected areas per green. In order to avoid the disease spreading, the visible spots/rings that were appearing were sprayed with a chemical fungicide. It was used fenarimol, metiram, pyraclostrobin and chlorothalonil, all compatible with the *Trichoderma harzianum* inoculation according to the commercial product instructions.

Along the experimental work it was monitored the soil temperature, since this golf course has several trees and other vegetation which might influence the micro climate among the chosen greens. Temperature was monitored and recorded at three moments in the day: in the morning (07:00 – 07:30h), at noon (12:30 – 13:00h) and at the afternoon (15:30 – 16:00h).

The data (number of spots/rings and affected area, both per area) were statistically evaluated by the t-Student test ($p<0.05$) using the SPSS ver. 15.0 software (Copyright (c) SPSS, Inc., 1989 - 2006).

2.2 *Trichoderma harzianum* concentration in the commercial product named Rizoderm (LIDA QUÍMICA, Spain)

The commercial product, Rizoderm was tested in order to evaluate the concentration of *Trichoderma harzianum*, grown in a semi-selective medium, and verified if the observed concentration value is in accordance with the specified by the manufacturer (LIDA QUÍMICA, Spain). Different dilutions of the product were inoculated in a semi-selective growth medium for *Trichoderma*, described by Chung and Hoitink [19]. Dilutions of the commercial product were prepared using deionized water to which a surfactant agent (tween) was added. One hundred µL of each dilution were inoculated at the surface of the agar plates containing the semi-selective growth medium and spread with a Drigalsky loop. Inoculated plates were incubated at aerobic conditions at 25 ± 1°C and kept until seven days. All tests were performed in triplicate.

2.3 Isolation of *Trichoderma harzianum* from soil and turfgrass samples

Throughout the experimental work and before each field application of the product, eighty (80) samples of soil + turfgrass were taken for the laboratorial detection of the presence of the antagonistic fungi *Trichoderma*.

Samples from the turgrass field were collected by means of a sterile inoxidable steel cylinder with fifteen (15) mm of diameter at fifty (50) mm soil depth.

2.4 Capability of *Trichoderma harzianum* to colonize the soil - laboratorial trial

To implement this study, three greens were selected from the experimental field; in each one three cylindrical samples of soil + turfgrass were randomly collected. These samples were collected by means of a sterile inoxidable steel cylinder with fifty (50) mm of diameter at fifty mm soil depth (Fig. 5).

From these samples the search of the presence of *Trichoderma harzianum* was conducted. After confirming the no presence of *Trichoderma* in the soil + turfgrass samples, the nine cylindrical samples were collected and inoculated with half of 10 $kg.ha^{-1}$ (T10) of the commercial product containing *Trichoderma harzianum*. The remaining product application was spread after 20 days. In this laboratorial trial the main objective was to observe the *Trichoderma harzianum* soil + turfgrass colonization capability.

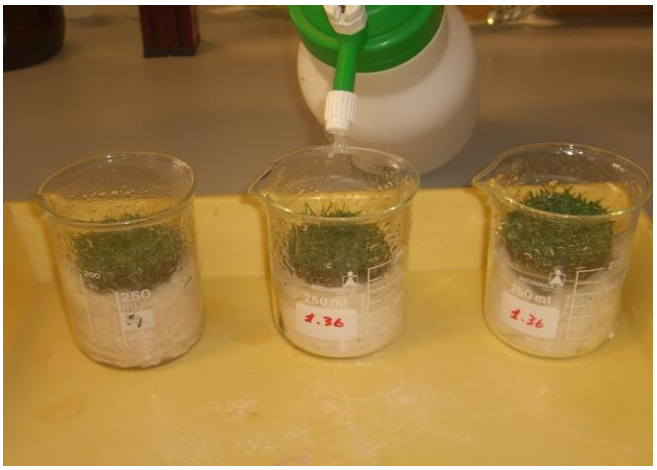

Fig. 5. Laboratorial trial with *Trichoderma harzianum* inoculation from the commercial product

2.5 Isolation of pathogenic turfgrass fungi

When and where symptoms of disease occurred, in the turfgrass, additional samples were collected as above mentioned (2.3). In these cases, samples were submitted to a prior chemical disinfection. Turfgrass samples were washed with a sodium hypochlorite solution, prepared from commercial bleach diluted in tap water to 0.5%, for 5 minutes and then washed with distilled water and dried with absorbent paper. The aim of this procedure was to reduce the microorganisms existing outside the plant material [20].

Different dilutions of the samples collected in the experimental field were inoculated in Petri dishes with a non selective culture medium for fungi: Potato Dextrose Agar (PDA, Biokar Diagnostics, Beauvais, France) and modified Sabouraud Dextrose Agar (Difco) supplemented with Neomycin and Polymicin [21].

One hundred µL of each dilution were inoculated at the surface of the agar plates containing the growth medium and spread with a Drigalsky loop.

Inoculated plates were incubated at aerobic conditions at 25 ± 1°C and kept until eleven days. All tests were performed in triplicate.

2.6 Pathogenic and antagonistic fungi morphological characteristics

To observe the morphological characteristics of the pathogenic and antagonist fungi isolated, extemporaneous preparations mounted with lactofenol (Panreac Quimica, S.A.U.) were done. The recognition of the different fungi was done by visual search of different features inherent in each fungus in

the growth media and after microscope observation. The survey took into account the symptoms observed in the turfgrass. Type cultures were also used to compare mycelium morphological and growth characteristics.

The microscopic preparations were observed by means of LEICA DMLB microscope; pictures were done by a LEICA MPS60 camera.

2.7 Maintenance of fungi isolates

Isolated colonies from pure cultures grown in Potato Dextrose Agar growth media were kept, at room temperature, protected from light, in Eppendorfs tubes containing 250 μL of sterile distilled water.

2.8 *Trichoderma harzianum* antagonistic activity study

In order to understand the *Trichoderma harzianum* antagonistic mode of action in the presence of pathogenic fungi, it was conducted a laboratorial experiment in a agar plate with a non selective growth medium (PDA). Thus, it was put on the surface of the agar plate a turfgrass clip and at one end of the vegetal material it was inoculated the antagonistic fungus (*Trichoderma harzianum*) and at the opposite end the pathogenic fungus *Fusarium* sp..

3 Results

3.1 Field experimental work

Soil temperature did not differ significantly from site to site, at the same hour of the day, among the experimental different blocks. Average soil temperature ranged from 17.9 to 26.1 ºC, along the day (Table 2).

Table 2. Average soil temperature (ºC) in the experimental work at three moments of the day (**M**, morning – 7:00h; **N**, noon – 12:30h; afternoon, **AN** – 16:00h)

Day	M	N	AN
30-May	18.1	24.3	25.0
31-May	17.9	23.8	24.7
01-Jun	18.1	24.5	26.1
06-Jun	19.4	22.3	23.2
11-Jun	18.1	20.4	22.9
15-Jun	18.5	20.3	22.3
20-Jun	18.1	21.3	23.4
25-Jun	19.6	22.2	24.8
27-Jun	18.2	23.9	25.7
03-Jul	20.1	23.7	26.1

The first symptoms of disease occurred five days after the beginning of the experimental work. Symptoms disease appeared especially on the untreated blocks; brown patches and fairy rings were observed in 7 of the 9 untreated blocks and only in one block of each rated treated blocks had symptoms of disease. Table 3 shows the affected areas and the number of spots/rings occurred.

Based on the cultural and morphological characteristics the isolated fungi were identified as *Rhizoctonia* (Fig. 6 and 7) and *Lepista* (Fig. 8 and 9), commonly called brown patch and fairy ring, respectively.

Among the nine blocks where it were observed disease symptoms, in three (2 from the untreated blocks, and one from the T15 treatment) had simultaneously both fungi disease symptoms.

During the experimental work, pathogenic fungi were isolated from 78.8% of the no treated blocks (T0); in 33.3% of the treated blocks (T10 and T15) the presence of pathogenic fungi were observed. The statistical treatment of the data based on the percentage of the affected area did not show significant differences ($p=0.659$) between treatments. Although, the number of spots observed in the control treatments (T0) was higher than the number observed in the treatments were *Trichoderma harzianum* was applied (Table 3).

Table 3. Presence of pathogenic fungi: affected area and number of spots/rings. T0 – control treatment; T10 – 10 $kg.ha^{-1}$; T15 – 15 $kg.ha^{-1}$

Treat	Number of spots	Affected area (%)	Observations (number of spots /rings)
T0	3	0.029	3 of *Rhizoctonia*
T0	7	1.889	7 of *Lepista*
T0	0	0.000	
T0	6	0.185	6 of *Rhizoctonia*
T0	2	0.354	2 of *Lepista*
T0	5	0.261	4 of *Rhizoctonia* + 1 of *Lepista*
T0	9	0.429	8 of *Rhizoctonia* + 1 of *Lepista*
T0	2	1.508	2 of *Lepista*
T0	0	0.000	
T10	0	0.000	
T10	3	0.334	3 of *Lepista*
T10	0	0.000	
T15	0	0.000	
T15	3	0.692	2 of *Rhizoctonia* + 1 of *Lepista*
T15	0	0.000	

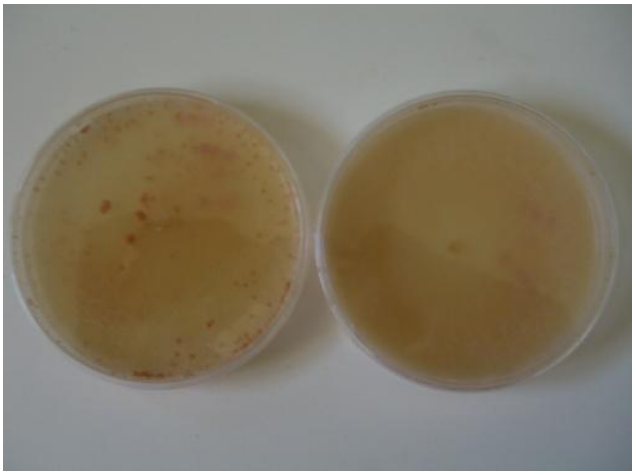

Fig. 6. *Rizhoctonia* sp. grown in Potato Dextrose Agar (PDA) culture media, after 7 days

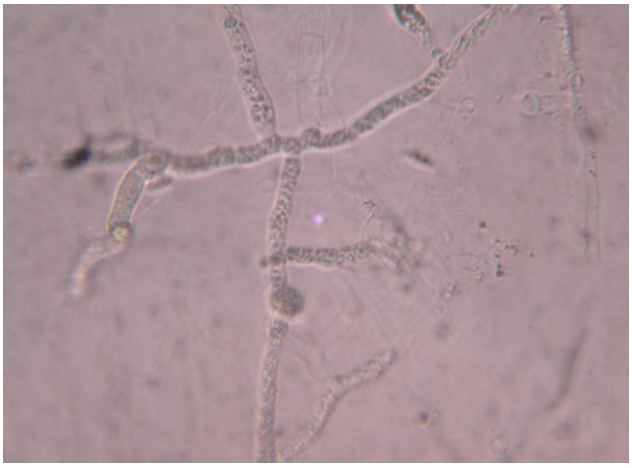

Fig. 7. Mycelium of *Rhizoctonia* sp. with typical T-form hyphae (1000x)

Fig. 8. *Lepista* sp. grown in Potato Dextrose Agar (PDA) culture media, after 11 days

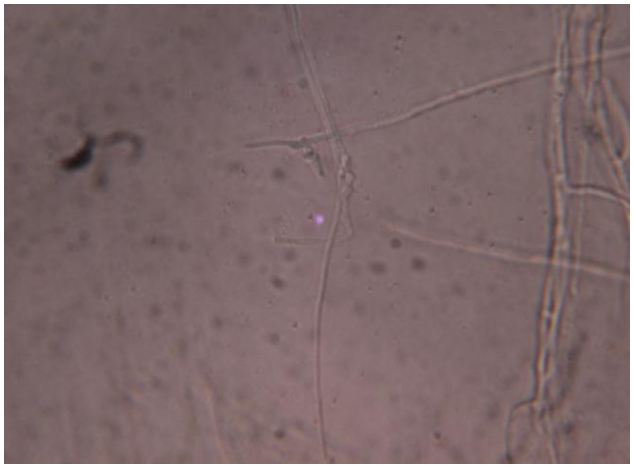

Fig. 9. Mycelium of *Lepista* sp. with the typical clamp connections (1000x)

3.2 *Trichoderma harzianum* concentration in the commercial product Rizoderm

This trial had as a main objective to know the concentration (cfu per gram) of *Trichoderma harzianum* in the commercial product. Our results showed values about one order of magnitude below the ones labeled on the product box (10^8 cfu g^{-1}).

3.3 Isolation of pathogenic fungi and *Trichoderma harzianum* from soil + turfgrass samples

Trichoderma harzianum was isolated (Figs. 10 and 11) in samples collected 20 days after the inoculation of the commercial product to the soil.

Considering the pathogenic fungi, *Rhizoctonia* sp. and *Lepista* sp. were the most frequent isolates. These fungi are responsible for brown patch and fairy ring turfgrass disease, respectively.

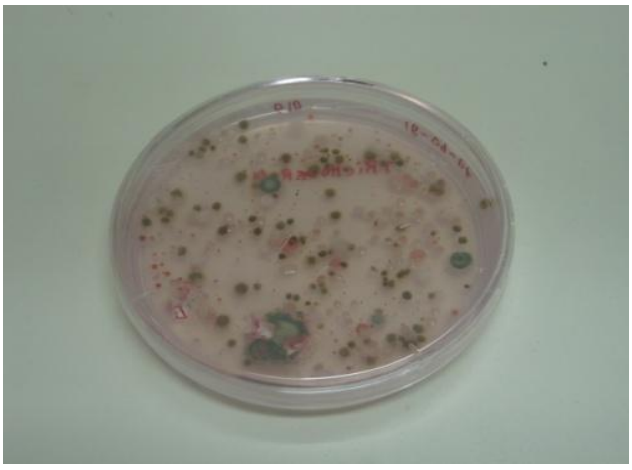

Fig. 10. *Trichoderma harzianum* grown in semi-selective media [18], after 7 days

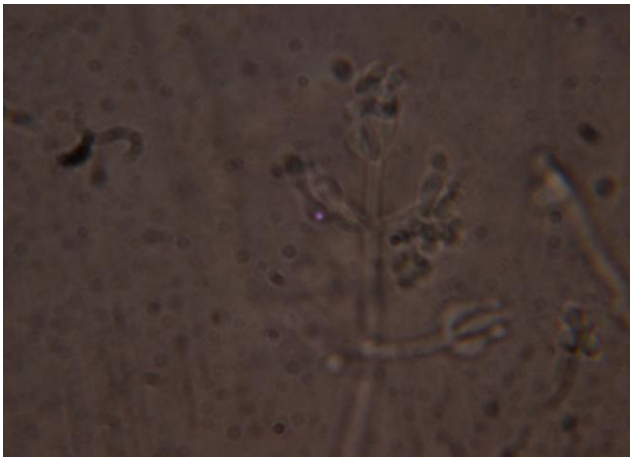

Fig. 11. *Trichoderma harzianum* recovered from the inoculated soil (1000x)

Sclerotinia sp. (dollar spot) and *Fusarium* sp. were also observed, however in other greens not belonging to the experimental area. The specific fungus of each disease were also isolated and identified in laboratory (Figs. 12 and 13), respectively, from the soil + turfgrass samples collected. However, *Fusarium* sp. was isolated from an area that did not show foliar disease symptom.

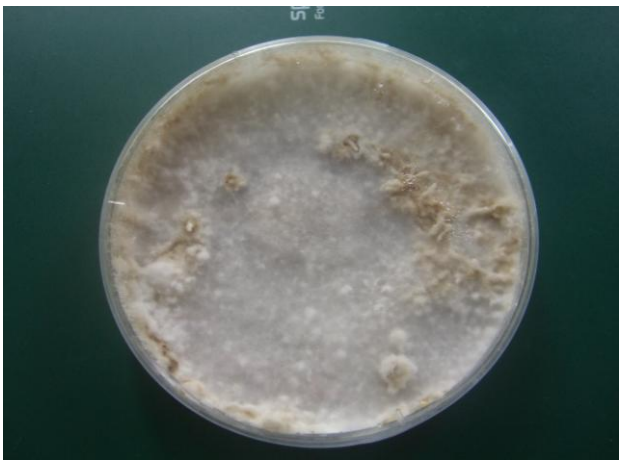

Fig. 12. *Sclerotinia* sp. grown in Potato Dextrose Agar (PDA) culture media, after 11 days

3.4 Capability of *Trichoderma harzianum* to colonize the soil - laboratorial trial

In the soil + turfgrass cylindrical (50 mm diameter; 50 mm depth) samples collected from the experimental field it were not detected *Trichoderma* before the commercial product inoculation. After 20 days of the first half inoculation, *Trichoderma harzianum* was detected in all nine samples. After 10 days of the second commercial product spreading, it was once more observed the presence of the antagonistic fungi *Trichoderma*.

With this trail it was achieved that the *Trichoderma harzianum* presence observed in the field was due to the spreading of the commercial product.

3.5 *Trichoderma harzianum* antagonistic mechanisms

The effect of the antagonist activity of *Trichoderma harzianum* against *Fusarium* sp. was slightly demonstrated in this trial (Fig. 13). The shape of the *Fusarium* sp. colony inflection suggests that a metabolite produced by *Trichoderma harzianum* was diffused in the growth media. The activity of *Trichoderma harzianum* against several pathogenic turfgrass fungi should be evaluated by means of other inoculation techniques. Other authors had demonstrated the antibiotic production of antibiotics including compounds affecting the integrity of fungal membranes and production of such fungal cell wall-degrading enzymes as chitinases [22].

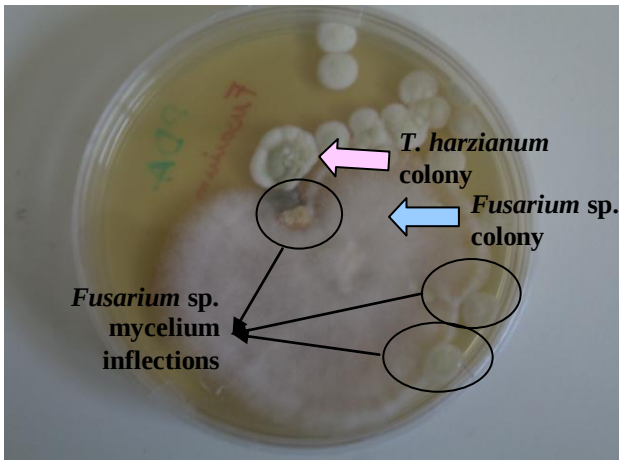

Fig. 13. *Fusarium* sp. grown in Potato Dextrose Agar (PDA) culture media, after 11 days

4 Conclusion

Trichoderma isolates are known for their ability to control plant pathogens. It has been shown that various isolates of *Trichoderma*, including *T. harzianum* isolate T-39 from the commercial biological control product TRICHODEX, were effective in controlling anthracnose (*Colletotrichum acutatum*) and grey mould (*Botrytis cinerea*) in strawberry, under controlled and greenhouse conditions [23].

This work showed that *Trichoderma harzianum* has the ability to control turf grass disease caused by *Rhizoctonia* sp. and *Lepista* sp..

This experimental work was short in time; experimental work concerning *Trichoderma* frequency spreading/inoculation must be carried out to study the antagonistic effect along the all season; [5, 7] showed that single application of compost did

not provide significant suppression of *Fusarium*, *Typhula* or *Sclerotinia*; these authors refer that compost application frequency of three weeks were effective in the reduction of dollar spot severity compared to the untreated control.

The diagnosis of turfgrass diseases requires substantial experience on recognizing field symptoms and on the identification of morphological mycelium structures at laboratorial fungal cultures. Microscopic observation of the pathogen is a good approach for disease diagnosis allied to foliar and/or root macroscopic symptoms.

More research should be done in order to study the level of efficacy and the reliability of this biocontrol approach that could be used as an alternative to the chemical fungicides. Studies of synergistic activity of the biocontrol agent and chemical fungicides should also be conducted in future research.

ACKNOWLEDGMENT

The authors are thankfully to the technical support given by the Sopantec and Lida Quimica (Pedro Sebastião and Carlos Leon, respectively); and to the Silves Golf (Miguel Grosso and Elisabete Eugénio) for the ease of access of the areas where the experimental field work was carried out.

References:

[1] Hardebeck, G. A., Turco, R. F., and Reicher, Z. J., Aplication of *Pseudomonas aureofaciens* Tx-1 through irrigation for control of dollar spot and brown patch on fairway-height turf. *Hortscience*, 39 (7), 2004, pp: 1750-1753.

[2] Beard, J. B., Turf Management for Golf Courses, 2nd Edition, Ann Arbor Press, Chelsea, Michigan, 816 pages, 2001.

[3] Tani, T., and Beard, J. B., Color Atlas of Turfgrass diseases. Ed. John Wiley and Sons, Inc. Hoboken, New Jersey. 245 pages, 1996.

[4] Kenna, M. P., and Snow, J. T., Fate and Management of turfgrass chemicals. ACS *Symposium Series*, 743, 2000, pp: 2-35.

[5] Boulter, J. I., Boland, G. J. and Trevors, J. T., 2002. Evaluation of Composts for Supression of Dollar Spot (*Sclerotinia homoeocarpa*) of Turfgrass, *Plant Disease*, 86: 405-410.

[6] Nelson, E. B., 1991, Introduction and establishment of strains of Enterobacter cloacae in golf course turf for the biological control of dollar spot. *Plant Disease*, 75, pp: 510-514.

[7] Boulter, J. I., Boland, G. J. and Trevors, J. T., 2002. Assessment of compost for suppression of Fusarium Patch (*Microdochium nivale*) and Typhula Blight (*Typhula ishikariensis*) snow molds of turfgrass. *Biological Control*, 25: 162-172.

[8] Nelson, E. B., Microbial strategies for the biological control of turfgrass diseases. *Fate and management of turfgrass chemicals*, 743, 2000, pp: 342-352.

[9] Ulhoa, C. J., and Peberdy, J. F., Regulation of chitinase synthesis in *Trichoderma harzianum*. *Journal of General Microbiology*, 137, 1991, pp: 2163-2169.

[10] Lima, H. J. L., Ulhoa, D. J., Fernandes, A. P., and Felix, C. R., Purification of a chitinase from *Trichoderma* sp. and its action on *Sclerotinia rolfsii* and *Rhizoctonia solani* cell walls. *Journal of General and Applied Microbiology*, 43 (1), 1997, pp: 31-37.

[11] Almeida, F. B. D. R., Cerqueira, F. M., Silva, R. D. N., Ulhoa, C. J., and Lima, A. L., Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: evaluation of coiling and hydrolytic enzyme production. *Biotechnology Letters*, 29 (8), 2007, pp: 1189-1193.

[12] Rosado, I. V., Rey, M., Codon, A. C., Gocantes, J., Moreno-Mateos, M. A., and Benitez, T., Cell wall protein *Trichoderma harzianum* is involved in cell protection and adherence to hydrophobic surfaces. *Fungal Genetics and Biology*, 44 (10); 2007, pp: 950-964.

[13] Savoie, J. M., and Mata, G., *Trichoderma harzianum* metabolites pre-adopt mushrooms to *Trichoderma* aggressivum antagonism. *Mycologia*, 95 (2), 2003, pp: 191-199.

[14] Hermosa, M. R., Grondona, I., Iturriaga, E. A., Diaz-Minguez, J. M., Castro, M., Monte, E., and Garcia-Acha, I., Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp.. *Applied Environmental Microbiology*, 66, 2000, pp: 1890-1898.

[15] Mocioni, M., Chemical and biological control of the most important turfgrass diseases in Italy. *Proceedings of the 1st International Conference on turfgrass management and science sports fields* (661), 2004, pp: 515-519.

[16] Balicevic, R., Paradikovic, N., and Samota, D., Control of soil parasites (*Pythium debaryanum*, *Rhizoctonia solani*) on tomato by a biological product. *Cereal Research Communications*, 35 (2), 2007, pp: 1001-1004.

[17] Jeffries, P. and Young, T.W.K., Interfungal parasite relationships. Cambridge University Press, 236 pages, 1994

[18] Silva, R. N., da Silva, S. P., Brandão, R. L., and Ulhoa, C. J., Regulation of N-acetyl-beta-D-glucosaminidase produced by *Trichoderma harzianum*: evidence that cAMP controls its expression. *Research in Microbiology*, 155 (8), 2004, pp: 667-671.

[19] Chung, Y. R., Hoitink, H. A. J., Interactions between thermophilic fungi and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in a bark compost-amended container medium, *Phytopathology*, 80,1990, pp: 73-77.

[20] Piernas, V. and Guiraud, J.P., Disinfection of Rice Seeds Prior to Sprouting. *Journal of Food Science*, 62 (3), 1997, pp: 611-615.

[21] Cohen, S., Modified Sabouraud Medium Containing Neomysin and Polymyxin, *Applied Microbiology*, 17 (3), 1969, pp: 486-487.

[22] Savoie, J.M. and Mata, G., *Trichoderma harzianum* metabolites pre-adapt mushrooms to *Trichoderma aggressivum* antagonism, *Mycologia*, 95(2), 2003, pp. 191-199.

[23] Freeman, S., Minz, O., Kolesnik, I., Barbul, O., Zveibil, A., Maymon, M., Nitzani, Y., Kirshner B., Rav-David, D., Bilu, A., Dag, A., Shafir, S., and Elad, Y., *Trichoderma* Biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* and Survival in Strawberry. *European Journal of Plant Pathology*, 110 (4), 2004, pp: 361-370.