Use of organic composts to suppress bentgrass diseases diseases in *Agrotis stolonifera*

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ABSTRACT

Turfgrasses are affected by fungal diseases and their chemical control is currently limited by the legislation and the impact on the environment of fungicides and the possible development of fungicide resistance. This study was carried out during three years to evaluate the antagonistic effect of the microbiota associated with organic composts. Two composts were produced from agro-industrial residues, P1 and P2. Fungi from both composts were isolated and identified and their antagonistic capacity was evaluated, *in vivo* and *in vitro* tests, against *Sclerotium rolfsii* Sacc, *Clarireedia* gen. nov. (formerly *Sclerotinia homoeocarpa* (Benn, 1937)) and *Rhizoctonia solani* (Kühn 1858). *In vivo* tests were run for *Agrostis stolonifera* L., seeded in P1, P2 and a commercial peat (T) based substrate (Hansa Torf Floragard, Germany), in 100 mL pots. A part of each substrate was submitted to a thermic treatment, obtained Pt, P1t and P2t substrates. Antagonistic properties of the P, P1 and P2 were compared with substrates

with thermic treatment. In the *in vitro* tests *Trichoderma atroviride* and *Trichoderma asperellum* showed higher growth than the tested pathogenic agents. *T. atroviride* showed the best antagonistic capacity, with inhibition percentages of 67.5%, 63.8% and 62.5% against *Clarireedia* spp., *S. rolfsii* and *R. solani*, respectively. In the *in vivo* tests, the efficacy and biological control index (BCI) were higher in P1 and P2 than in P. BCI ranged from 1.66 (Pt) to 39.03 (P2) on *S. rolfsii* control, from 1.38 (Pt) to 14.66 (P1) on *Clarireedia* spp. control, and from 1.87 (Pt) to 21.45 (P1) for *R. solani* control. Compost P2 showed the highest suppressive effect on the studied soil diseases in turfgrass, which did not occur when its microbiota was eliminated by thermic treatment.

Keywords: best management practices; nitrogen; plant disease; turfgrass management

1. Introduction

Composting is a solid waste treatment method, where organic components are biologically decomposed under controlled conditions, achieving a state in which it can be handled, stored or applied to the soil without affecting the environment (Golueke, 1991). The composting process is affected by physical and chemical characteristics such as temperature, aeration, humidity and C/N ratio (Boulter et al., 2000; Day and Shaw, 2005).

In the Algarve region (south of Portugal), golf is an economically important activity with both direct and indirect incomes. In this region of Portugal there are about 40 golf courses, with an estimated area of 2 000 ha of turfgrass. A correct and sustainable maintenance of the lawns is required, considering that golf lawns are quite susceptible to foliar-and soil-borne diseases (Barker, 2001; Saharan and Mehta, 2008), which causes aesthetic disorders and loss of greens playability and are responsible for economic

losses. The use of synthetic fungicides to control plant pathogens has caused several problems, such as fungal resistance, ecosystem imbalance by toxic effects of residues and human and animal health hazards (Johnson and Atallah, 2006). The use of organic composts can contribute to reducing imbalances in golf course ecosystems.

The increasing interest regarding environment and public health protection enhanced the search for alternatives, leading researchers to seek strategies to reduce the use of synthetic products, such as fertilizers and pesticides (Bonanomi et al., 2010). Also, the European (Directive 2009/128/EC, of October 21st) and national legislation (Portuguese Law 26/2013, of April 11th) is directing the reduction of pesticides (Trillas et al., 2006).

Organic composts demonstrated a natural suppressive capacity concerning plant pathogens, namely *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Phytophtora* spp., *Ralstonia solanacearum* in different plants (Cotxarrera, et al., 2002; Pugliese et al., 2008; Borrero et al., 2009; Alfano et al., 2011; Castaño et al., 2011; Castaño et al., 2013; Jambhulkar et al., 2015; Liu et al., 2016). Disease supression is a process that depends on microbial community structure and increased competitive ability of beneficial microorganisms against pathogens (Liu et al., 2016).

The use of antagonistic microorganisms is an alternative and an attractive approach for disease control, which has proved to be economically sustainable and environmentally compatible, without the negative impact of chemicals (Barker, 2001; Stowell and Gelernter, 2001; Trillas et al., 2006; Dempsey et al., 2012; Gan and Wickings, 2017). Biocontrol agents are easy to deliver, may activate plant resistance mechanisms and contribute to improve plant growth.

Trichoderma spp. is a biological control agent, that has been successfully studied in numerous crops, against different plant diseases (Trillas et al., 2006, Rossi and Pattori, 2009, Huang et al., 2011, Coelho et al., 2016, Hirpara et al., 2016).

Turfgrass are susceptible to a variety of fungal soil pathogens, being *Sclerotium rolfsii*, *Clarireedia* spp. (identified as *Sclerotinia homoeocarpa* by Benn (1937) and *Rhizoctonia solani* among the most frequent. *Sclerotinia* spp. symptoms may persist from early summer until early fall (Saharan and Mehta, 2008); and *R. solani* cause a serious disease in the warm humid and warm tropical climatic zones (Burpee and Martin, 1992). Turfgrass topdressing with compost, alone or mixed with sand, may increase the soil microbial activity, and consequently the suppressive capacity of several turfgrass diseases. In this three-year study, the suppressive capacity of two organic composts, produced from agroindustrial wastes were studied. Potentially antagonistic fungi were identified and their antagonistic effect was evaluated *in vitro*. To evaluate the suppressive capacity of the produced composts, bentgrass (*Agrostis stolonifera* L., var. T1, the main specie used on golf courses greens) was sown using these two organic composts as substrate and their supressivity for soil diseases was tested. Disease incidence, number of disease spots, affected area by the disease, disease severity, area under disease progress curve (AUDPC), percentage of mean disease reduction and Biological Control Index (BCI) were evaluated.

2. Material and Methods

The experiments were carried out at the *Campus* of Gambelas, University of Algarve, Portugal (37°02'35.45"N, 7°58'20.64"W).

2.1. *Organic composts production*

Two composts were produced, P1 and P2, by composting agro-industrial residues in two piles with aeration by turning. P1 compost was obtained with a mixture of orange fruit waste from a farmer's association, grass clippings from golf courses and grape

101 marc from a wine house (0.9:1.1:1 v/v). P2 compost was produced with the same raw
102 materials, to which waste from mushroom production (*Shiitake*) was added
103 (1.8:0.2:1.5:0.5 v/v). Mixes were prepared to obtain the similar initial C/N ratio in both
104 piles. Initially volume of each composting pile, P1 and P2, was about 1 m^3. The piles
105 were protected with a geotextile from rain and direct sun. Composting was repeated
106 three consecutive times. During the composting processes, temperature was measured
107 daily, in four different places in the piles, with a portable probe (TP 62, Umwelt
108 Elektronik GmbH & Co.KG, Germany) that measured the temperature in 6 different
109 points, distanced 20 cm along the probe. pH was determined in an aqueous extract (1:2
110 w/v) with a potentiometer (Crison Micro pH, 2001), and the electrical conductivity was
111 measured in the same extract, after filtration, with a conductivity meter (Crison 522).
112 The organic and mineral content (Ramos et al., 1987), dry matter (Martinez, 1992), C/N
113 ratio, nitrogen and microbiological populations were also assessed. The Zucconi index
114 of the composts (Zucconi et al., 1985), which indicates the absence of phytotoxic
115 substances, was evaluated following a germination test of *Lepidium sativum* L. Zucconi
116 index value below 65-70% indicate the possible presence of toxic substances. The
117 microbial tests were carried out by the enumeration of fungi and bacteria in adequate
118 culture media. Samples were suspended in phosphate buffered saline (PBS) and serial
119 decimal dilutions were prepared and then inoculated, into a culture media suitable for
120 the tested microorganism growth. For fungi, aerobic bacteria, actinomycetes and
121 chitinolytic microorganisms enumeration, culture media were inoculated by the spread
122 plate technique. The enumeration of the cellulolytic microorganisms, occurred in liquid
123 medium and so the Most Probable Number technique (FDA, Bacterial Analytical
124 Manual, Appendix 2) was used.

125 Fungi were cultivated on Potato Dextrose Agar (PDA) (Biokar, France) plates and
126 incubated at 25 ± 2 ºC and 55 ± 2 ºC for 24-48 hours; total aerobic bacteria, on Plate
127 Count Agar (PCA) (Oxoid, England), at 25 ± 2 ºC and 55 ± 2 ºC for 24-48 hours;
128 actinomycetes, on 1/2PCA at 25 ± 2 ºC and 55 ± 2 ºC for 24-48 hours (Coelho et al.,
129 2013); chitinolytic aerobic microorganisms were grown on Mineral Salt Medium
130 (MSM) added with chitin and agar, at 28 ± 2 ºC for 5 days (Alfano et al., 2011).
131 Cellulolytic aerobic microorganisms were cultivated in MSM with 0.5 x 8.0 cm strips of
132 filter paper as a carbon source, at 37 ± 2 ºC for 15 days (Alfano et al., 2011). These
133 assays were carried out in triplicate.
134 At the end of the composting processes, after 112 days, physical properties of the
135 composts were determined, namely water retention curve and bulk density (De Boodt et
136 al., 1974).
137
138 *2.2. Isolation and Identification of Biological Control Agents*
139
140 At the end of the thermophilic phase (cooling period) and at the end of the composting
141 process(between days 70 and 112), fungi were collected from both piles, grown in PDA
142 and isolated in pure culture . The first identification step was trough cultural
143 characteristics, followed by microscopy to identify the genera of the identified fungi
144 isolated from the composts. After the *in vitro* growth inhibition tests, the fungi that
145 showed an inhibition percentage above 50% (procedure described in the next section),
146 were identified by molecular techniques. The identification of fungi species was done
147 by analysis of nucleotide sequences of the ITS1-5.8S-ITS2 region using the primers
148 ITS1 and ITS4 (White et al., 1990). BLAST searches of GenBank showed a high

149 similarity of the isolate sequence. Fungi that showed antagonistic capacity were
150 maintained on PDA and Corn Meal Agar (CMA) medium.
151
152 *2.3. Screening antagonistic capacity - In vitro experiment*
153
154 The fungi isolated from the composts were tested *in vitro* for its antagonistic capacity,
155 by the direct confrontation technique (adapted from Dennis and Webster, 1971; Chen et
156 al., 2016) against *S. rolfsii*, *Clarireedia* spp. (from the Sevilla University) and *R. solani*
157 (from the Barcelona University).
158 According to the direct confrontation technique, one disc with active growing mycelium
159 of the pathogen and another with mycelium of the antagonist, were placed on the
160 surface of the culture media, 3 cm apart. Both mycelia were grown for seven days in
161 PDA. Each fungus was also cultured alone to determine its own growth rate. All the
162 assays were run in triplicate and were conducted three times. Inoculated plates were
163 incubated in the dark at 25 ± 2 ºC. Daily measurement of the radius of the growth zone
164 of each fungi was taken, to calculate the inhibition percentage (IP), according to $IP =$
165 $\frac{(Rc-R1)}{Rc} \times 100$, where Rc and R1 are the radius of the growth of the pathogen grown
166 alone and the radius of the growth of the pathogen in the presence of the antagonist,
167 respectively.
168
169 *2.4. Screening of the Antagonist capacity - In vivo experiments*
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171 To evaluate the suppressive capacity of P1 and P2 composts *in vivo*, turfgrass (*Agrostis*
172 *stolonifera* L. var. T1) was sown in 100 mL pots, in a non-heated greenhouse. Six
173 different treatments were setup: peat (P) (Hansa Torf Floragard, Germany), composts

P1 and P2, and the same substrates submitted to a thermic treatment (t) at 60 ºC for seven days, referred as Pt, P1t and P2t, respectively. Each treatment consisted of five pots, with four repetitions. When turfgrass covered all the surface of the pots, pathogenic agents *S. rolfsii*, *Clarireedia spp.*, and *R. solani* grown as pure culture in PDA for seven days, were inoculated. Inoculation was done by placing a 6.5 mm diameter PDA disk with active growing mycelia. In the assay with *S. rolfsii*, in addition to the disk with the mycelium, two *sclerotia* were also added to each pot. The three pathogens studied were inoculated simultaneously. The substrates submitted to thermic treatment and inoculated with the pathogen were used as positive control; substrates submitted to thermic treatment, but not inoculated, were the negative control. In order to increase and stabilize humidity and air temperature, the pots were kept under a tunnel inside the greenhouse. Air temperature and relative humidity near the plants were daily recorded. Plants were irrigated by fine micro sprinkler irrigation, to maintain the environmental high humidity. Foliar fertilization with nitrogen (18% N and 8% organic matter) was weekly applied using an organic-mineral fertilizer (Ret-Sul, Eibol, Spain), at the manufacturer recommended concentration of 150 mL $100L^{-1}$, corresponding to 4.5 kg nitrogen ha^{-1} Turfgrass was mow weekly with a shears.

The greenhouse experiment were conducted three times, with eight to ten months interval. For each trial a new compost was prepared using the same material mix and composting technique. The number of disease spots and the affected area by each disease was recorded weekly in all the pots, for disease severity and disease incidence determinations. Disease severity was scored on a symptom severity scale, adapted from Baayen and Van der Plas (1992), where: 0 = asymptomatic plant (0% disease); 1 = weakly infected plant (5% disease); 2 = local base-stem symptoms (20%); 3 = well developed symptoms (50%); 4 = strong disease symptoms throughout the area (80%); 5

= dead plants (100%). The area under disease progress curve (AUDPC) was determined according to Campbell and Madden (1990), ranging from 0 to 1 (showing an increasing speed of the disease development). AUDPC per pot was calculated by the disease severity integrated between the onset of symptoms and assay completion and dividing by the total of the epidemic duration (days) in each assay, in order to compare the various assays, which had a variety of epidemic durations, according to: $\sum_{i=1}^{n}\left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1}-t_i)$, where y = injury disease and *t* = time.

Biological control index (BCI) and efficacy was calculated according to Byrne et al. (2005). The BCI was applied to combine the efficacy-consistency of the disease reduction, according to: BCI = $\frac{Efficacy}{Consistency}$. The efficacy is the percentage of the mean disease reduction and the consistency is the standard deviation of those means.

2.5. Statistical analysis

Physical and chemical characteristics of P1 and P2 were compared with a Student's Ttest. For the comparison of the three substrates (P1, P2 and the commercial peat), multifactorial analysis of variance (ANOVA) followed by Duncan New Multiple-Range Test was used. Data from the inhibition percentage (*in vitro* trials) and from the *in vivo* experiments, such as disease incidence, number of disease spots, affected area by the disease, disease severity, AUDPC and BCI were analyzed by ANOVA, and compared using the Duncan New Multiple-Range Test. The normality of the sample distribution and the homogeneity of the variances were verified before ANOVA (Zar, 1999). Person's Correlation was used to test the significance of the relation between disease progress and disease reduction, and between disease progress and compost pH, EC,

organic matter and nitrogen content. For the statistical analysis, the software IBM SPSS Statistics ver. 25 (IBM Corp., 1989-2017, U.S.A.) was used.

3. Results and Discussion

3.1. Organic composts production

When comparing composts, according to a t's Students test, P2 presented the highest pH ($p < 0.001$), although both were in the neutral range; EC was significantly higher in P2 ($p<0.05$) as well as organic matter ($p<0.001$) (Table 1). Compost P1 presented highest nitrogen content ($p<0.05$), probably due to the higher proportion of grass clippings in the initial mix. However, nitrogen content in both composts was above the minimum recommended value of 0.75% (Ferreira et al., 2002). These small differences in compost characteristics might have had a minor influence on microorganisms' populations.

The mentioned characteristics (pH, EC and N content) favoured the development of large populations of microorganisms on both composts (Table 2), as report by Coelho et al. (2012), in similar composting processes. The microbial activity is responsible not only for the fast transformation of the organic residues during composting, but also for the development of supressive capacity in the composts.

At the end of the composting process, the highest microbiological populations were recorded in P2 compost, exception for the actinomycetes (Table 2). The different physical and chemical characteristics of P1 and P2 enhanced the development of the composts microbiota. Differences between the two studied composts related with pH, EC, organic matter and nitrogen content should be explained by their different chemical composition.

As expected, the addition of mushroom residues in P2, which are rich in chitin, with an average content of 57.75 mg g^{-1} (López-Mondéjar et al., 2012), promoted the growth of chitinolytic microorganisms (Table 2). The presence of chitin in the initial mix stimulates the development of fungi with the capacity to decompose chitin, by the action of chitinase (Sharp, 2013). The significant differences of organic matter content in the composts contributed to increase the number of fungi, bacteria and actinomycetes populations (Antil et al., 2013).

The germination test of *Lepidium sativum* L. showed significant higher germination rate ($p<0.001$) using P2 (91.8%) than P1 (85.2%) (Table 1). However, in both cases above the minimum required for their safe agricultural use, according to Zucconi et al. (1985), that indicate an acceptable minimum germination percentage of 65%.

3.2. Isolation and identification of the biological control agents

During the composting processes of P1 and P2, 190 fungi were isolated. During the cooling and maturation phases, 31 fungi with potential antagonistic effect, were identified: 14 from P1 and 17 from P2 (Table 2). The presence of chitin in P2 may have promoted the increased number of antagonists isolated as reported by Sharp (2013). Other properties, such as pH, electrical conductivity, organic matter and physical properties could also promote the antagonistic effect of P2 (Cotxarrera et al., 2002; Avilés et al., 2011; Pane et al., 2011).

The 31 potential antagonistic fungi isolated, were morphologicaly identified as *Trichoderma*, *Gliocladium*, *Penicillium*, *Aspergillus*, *Purpureocillium, Fusarium*, *Bionectria*, *Beauveria*, *Clonostachys* and *Metarhizium,* which are frequent in organic composts (Mehta et al., 2014).

By the analysis of nucleotide sequences, the 31 isolated fungi were putatively identified as: *Trichoderma atroviride, Trichoderma asperellum, Fusarium oxysporum, Fusarium keratrosplasticum, Fusarium lichenicola, Bionectria ochroleuca, Beuaveria bassiana, Clonostachys rosea* and *Maetarhizium anisopliae*. *T. asperellum, B. ochroleuca, B. bassiana* and *C. rosea* were isolated only from P2, and *F. keratrosplasticum* only from P1. One isolate of *T. atroviride*, one of *F. oxysporum*, one of *F. keratroplasticum*, one of *F. lichenicola* and one of *M. anisopliae* were isolated from P1 and showed the best antagonistic capacity. From P2, five isolates were identified as *T. atrovide*, one as *T. asperellum*, one as *F.oxysporum*, one as *F. lichenicola*, one as *M. anisopliae*, one as *C. rosea* and one as *B. ochroleuca* (isolated from the chitynolitic culture medium). Probably, *F. oxyporum* isolated from P1 and P2, were a non-pathogenic *Fusarium*, because no symptoms of fusarium wilt were observed in the tested plants. The great diversity of *Trichoderma* spp. can be explained by the high lignocellulosic substances from agricultural wastes (Jambhumlkar et al., 2015).

3.3. In vitro tests

In the *in vitro* tests, the inhibition percentage rates were significantly different among the antagonistic fungi ($p<0.05$).

3.3.1. *S. rolfsii*

Ten fungi (*Trichoderma* spp. and *Fusarium* spp.) showed a significant higher percentage of growth inhibition of *S. rolfsii*, with growth inhibition rates between 51 and 64% (Table 3). The highest inhibition percentage rate for *S. rolfsii* was observed

297 with *T. atroviride*. From these ten fungi isolates, seven were isolated from P2 and three
298 from P1 (Table 3).
299
300 3.3.2. *Clarireedia spp.* (formerly *S. homoeocarpa)*
301 Twelve isolated fungi reduced *Clarireedia* spp. growth (*Trichoderma* spp., *Fusarium*
302 spp. and *Bionectria* spp.). *Clarireedia* spp. suppression rates ranged between 52 and
303 68%. *T. atroviride* performed the highest inhibition percentage (68%). Eight of these
304 twelve fungi were isolated from P2 and four from P1.
305
306 3.3.3. *R. solani*
307 Fifteen fungi isolates (*Trichoderma* spp., *Fusarium* spp. and *B. ochroleuca*) reduced *R.*
308 *solani*, with growth inhibition rates between 50 and 63%. *T. atroviride* performed again
309 the highest inhibition reduction for this disease. Nine of these fifteen fungi were isolated
310 from P2 and six from P1.
311 The highest growth inhibition was achieved with *T. atroviride*, as observed by Lahlali
312 and Hijri (2010), when confronted *T. atroviride* with *R. solani* in potato plants. Some
313 fungi isolated from P1 and P2 inhibited the pathogenic fungi growth, and more
314 antagonists were isolated from P2. According to Castaño et al. (2011), the composting
315 system used and the composition of the raw material, may affect the suppressive
316 capacity of the organic composts, and may promote a different microbiological richness.
317 In P2, the presence of chitin, in the raw material, may have played a key role in the
318 microbiological diversity.
319
320 *3.4. In vivo tests*
321

322 Studied diseases (*R. solani*, *Clarireedia* spp. and *S. rolfsii*) showed a significant smaller
323 diseased area ($p < 0.05$) was measured in the pots with the organic composts,
324 particularly with P2 (Fig. 1, Fig. 2 and Fig. 3). The exception was for *Clarireedia* spp.,
325 where disease affected area was lower in P1 ($p < 0.05$) (Fig. 2). In substrates without
326 pathogenic agent inoculum (control) there were no symptoms of diseases. Concerning
327 *Clarireedia* spp., Walsh et al. (1999) reported that a method of control is the application
328 of nitrogen. P1 had higher nitrogen content ($p < 0.01$) (Table 1), which may have
329 contributed to the reduction of the affected area by this disease (Fig. 1). When compared
330 with peat (P), the microbiological populations in P1 and P2 contributed to an higher
331 suppressive disease capacity due to the microbiological activity (Hoitink et al, 1993;
332 Hoitink et al., 2001). When substrates were submitted to a thermic treatment (negative
333 control) disease affected area was larger for studied diseases (Fig. 1, Fig.2 and Fig.3),
334 with significant differences ($p<0.05$), because of the elimination of the microbial
335 populations in the composts (Borrero et al., 2006). At the end of the assay, turfgrass
336 grown on P1 and P2 showed significant less area affected by the diseases ($p<0.001$),
337 when compared to the other substrates. Compost role in the biological control was
338 clear. Comparing the six substrates studied, it was found that the diseases developed
339 faster in the substrates submitted to a thermic treatment and peat, showing the positive
340 effect of organic composts. Moreover, considering that diseases developed only on the
341 inoculated pots, being no symptoms observed in the pots without inoculum, it shows
342 that the origin of the disease was due to the inoculation and not by any other form of
343 contamination.
344 Composts have been reported to be good biological control agents concerning different
345 crops phytopathogenic fungi such as *Fusarium oxyporum* in tomato (Cotxarrera, et al.,
346 2002; Castaño et al., 2011; Castaño et al., 2013) and carnation (Borrero et al., 2009;

Castaño et al., 2011; Castaño et al., 2013), *F. oxyporum* and *Pythium ultimum* in tomato (Alfano et al., 2011) or *R. solani* in bean and *F. oxysporum* f.sp. *basilica* in basil (Pugliese et al., 2008).

The disease severity approached the maximum value (5) for *Clarireedia* spp. in P, Pt, P1t and P2t (Fig. 2). The diseases were less severe in P1 and P2, especially P2, in the case of *R. solani* (Fig. 1); P1 was the most suppressive compost to control *Clarireedia* spp. (Fig. 2). In this study, a negative correlation between disease severity and some compost properties was observed. The highest pH and EC of P2 had a negative effect on the growth of the pathogens. Cotxarrera et al. (2002) observed that higher pH and EC reduced the growth of *Fusarium oxysporum* in tomato, reducing the survival of the pathogen. Those authors referred that the highest values of pH reduced the growth, sporulation and pathogenicity of *F. oxysporum*, since it reduces the availability of micronutrients such as Fe, Cu and Zn. Pane et al. (2011) reported a negative correlation between plant damping-off by *S. minor* and salinity of the compost.

Disease incidence ranges from 0 to 1, indicating its absence or presence in the pots, respectively (Reis and Coelho, 2013). The disease incidence was significant lower in P1 and P2 ($p<0.05$), when compared with the other treatments. P1 compost stands out in the reduction of the incidence of *Clarireedia* spp., probably because of the higher nitrogen content (Walsh et al., 1999). A significant negative correlation was observed a between this nutrient and the affected area and disease severity ($p<0.001$)and incidence. Townsend et al. (2018) also reported that higher nitrogen levels reduced dollar spot severity. P2 stands out in the reduction of *R. solani*, probably due to its lower nitrogen content, as observed by Tani and Beard (1997) and Tredway and Burpee (2001). The best performance of P2 in *R. solani* control may also attributed to the higher population

371 of *Trichoderma*, which is known as a excellent *R. solani* antagonist (Hoitink et al.,
372 1991).
373 AUDPC used as a severity index, indicated that P1 and P2, compared to the substrates
374 submitted to a thermic substrates and peat, had higher diseases suppressive effect (Fig.
375 1, Fig. 2 and Fig. 3). Borrero et al. (2009) observed that in the control of Fusarium wilt
376 in carnation, the organic composts also showed smaller AUDPC when compared to
377 peat. For the studied diseases, at the end of the assays, AUDPC was significantly lower
378 in P1 and P2 ($p<0.05$), compared to the other substrates (Fig. 1, Fig. 2 and Fig. 3).
379 The BCI was significantly higher with P1 and P2 ($p<0.05$) (Fig. 1, Fig. 2 and Fig. 3).
380 BCI ranged from 1.66 (in Pt) to 39.03 (in P2) to control *S. rolfsii* (Fig. 3), from 1.38 (in
381 Pt) to 14.66 (in P1) to control *Clarireedia* spp. (Fig. 2) and from 1.87 (in Pt) and 21.45
382 (in P1) for *R. solani* (Fig. 1). Similar values were found by Suárez-Estrella et al. (2013),
383 in the control of *F. oxysporum* in tomato. When compared to the other five substrates,
384 P2 showed a higher BCI for *S. rolfsii* (Fig. 1, Fig. 2 and Fig. 3). A positive correlation
385 between microbial populations of the composts and BCI was found. Larger and more
386 diverse populations of microorganisms can contribute to the success of organic
387 composts in biological control, as described by Castaño et al. (2013), namely
388 *Trichoderma* sp.. In sugarbeet, Upadhyay and Mukhopadhyay (1986) observed that
389 *Trichoderma harzianum* attacked and lysed the mycelium and sclerotia of *S. rolfsii*.
390 Jambhulkar et al. (2015) reported that organic composts showed a suppressive capacity
391 against soil diseases, namely *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp.,
392 *Phytophtora* spp.. However, only 20% of the organic composts can reduce damping-off
393 caused by *Rhizoctonia* spp. (Hoitink and Boehm, 1999). *R. solani* is controlled by a
394 specific suppressive process by *Trichoderma* spp. (Hoitink et al., 1991). Both composts
395 (P1 and P2) exhibited a suppressive capacity for *R. solani* (Fig. 1), probably due to its

microbial populations, namely *Trichoderma* spp. A positive correlation was observed between *Trichoderma* spp. population and the BCI for *R. solani* (R^2=0.684, $p<0.01$). The physiochemical characteristics of the composts can affect suppressive properties through direct effects on pathogens and antagonistic microorganisms, or indirect effects on host systems through the supply of nutrients, improvement of soil structure, porosity and water retention capabilities, along with other factors (Boulter et al., 2000). The aeration capacity of P1 was 14.6 % but P2 had a significant higher aeration, 25.6% ($p<0.05$). The best biological control of P2 may be related to its aeration ability. According to Avilés et al. (2011), the aeration ability has a positive effect on the severity of rotting diseases of plant roots. Another reason for the higher biological control capacity of P2 may be the presence of chitin from the residues of mushroom, known to contain about 57.8 mg g^{-1} of mushroom residue (López-Mondéjar et al., 2012). Composting organic residues with chitin promotes the development of fungi with increased degradation capacity due to the action of chitinase, reinforcing the suppressive potential of the composts against pathogens with chitin in their structure, by the lysis of their cells (Alfano et al., 2011; Sharp, 2013). The presence of chitinolytic organisms is associated with the disease suppression mechanisms based on pathogen cell-wall hydrolysis, such as mycoparasitism and hyperparasitism.

High populations of actynomicetes also influence the suppressive ability of the composts (Mahatma and Mahatma, 2015). With the studied diseases, chitinolytic populations showed a negative correlation with the disease development parameters, contributing to their biological control.

4. Conclusions

The organic composts from agro-industrial wastes have characteristics that allow the development of antagonistic microorganisms against soil fungi pathogens, namely *Clarireedia* spp., *S. rolfsii* and *R. solani*. *Trichoderma atroviride*, that showed the best disease inhibition results: 56.4 to 67.5%, on the control of the three studied pathogenic fungi. Composts showed higher suppressive capacity. P1 against *Clarireedia* spp. and P2, with less nitrogen content and the highest *Trichoderma* spp. populations, showed the higher capacity to control *R. solani*. The results showed that the use of the tested composts has potential as a biocontrol cultural practice, reducing the use of pesticides in turfgrass management.

Studies will continue, It will be also important to evaluate the effect of compost enrichment with antagonists, and determine the effect of the storage period on the efficacy of the compost.

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Tables and figures

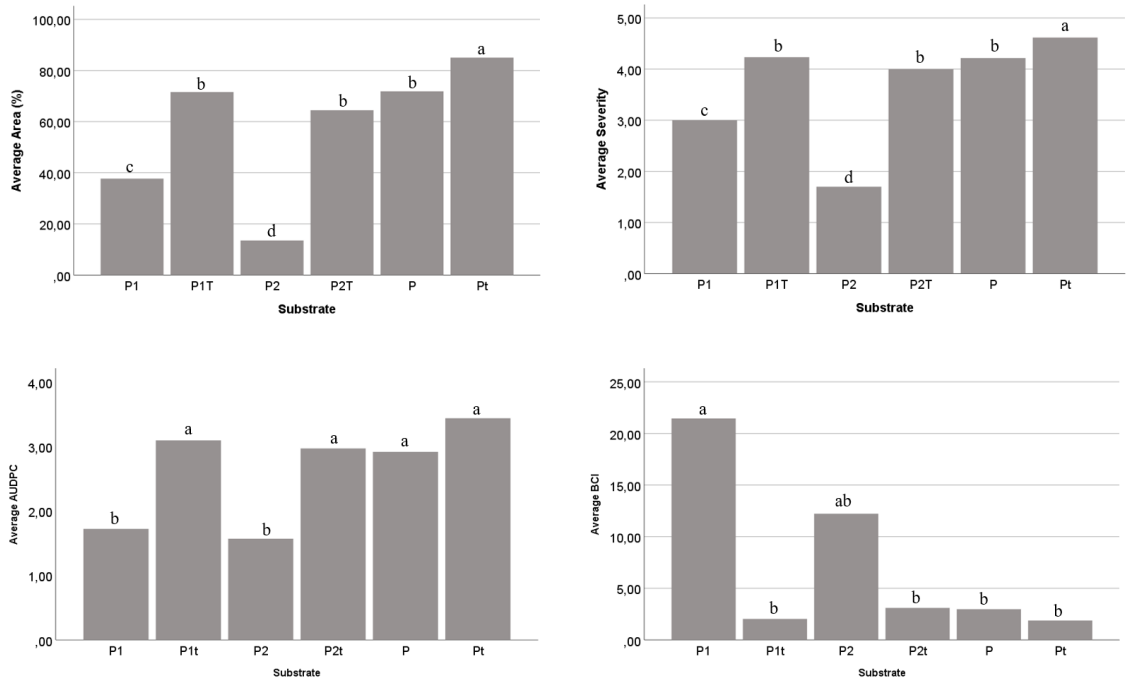
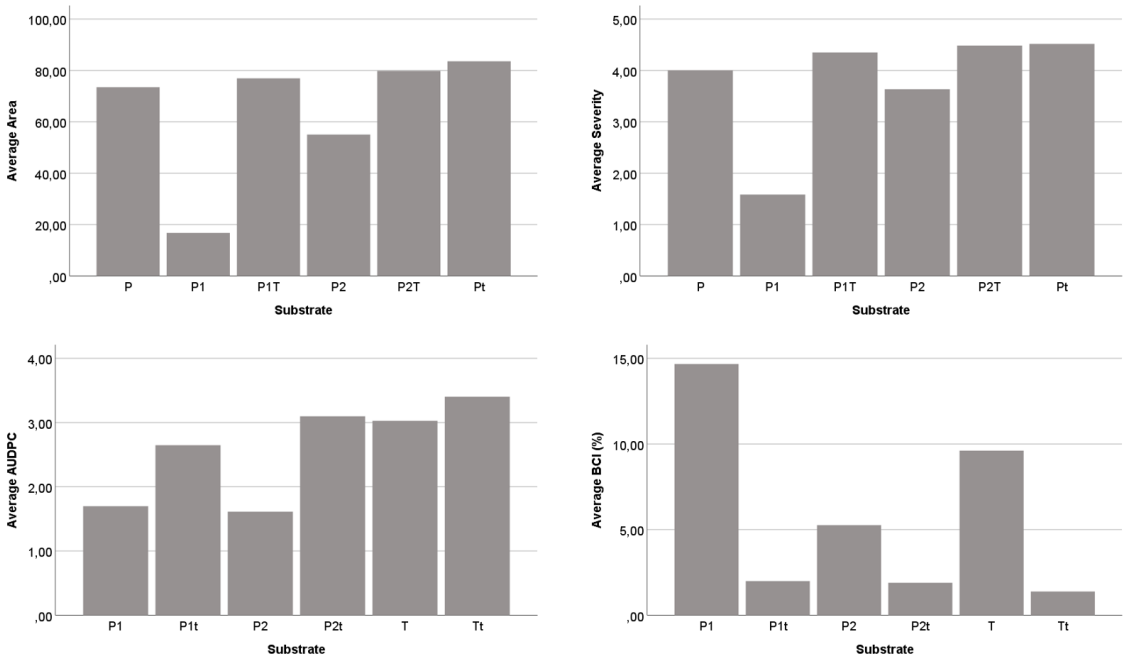


Fig. 1. Compost effectiveness in the biological control of the *Rhizoctinia solani*. Average area, average of the affected area by disease; Average Severity, average of the disease severity; Average AUDPC, average of the area under disease curve progress; Average BCI, average of the biological control index; P1, compost P1; P1t, compost P1 with thermal treatment; P2, compost P2; P2t, compost P2 with thermal treatment, P, peat; Pt, peat with thermal treatment. Bars depicting the same character have no statistically significant differences for $p<0,05$ (Duncan test).



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652 Fig. 2. Compost effectiveness in the biological control of the *Clarireedia spp.*. Average
653 area, average of the affected area by disease; Average Severity, average of the disease
654 severity; Average AUDPC, average of the area under disease curve progress; Average
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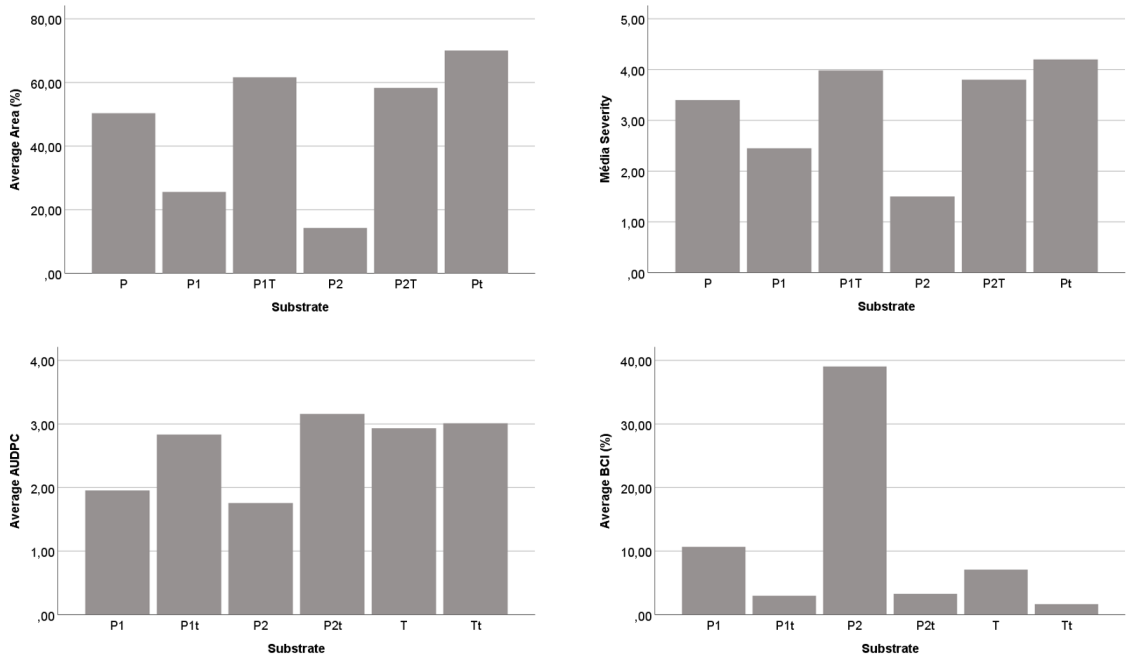

Fig. 3. Compost effectiveness in the biological control of the *Sclerotium rolfsii*. Average area, average of the affected area by disease; Average Severity, average of the disease severity; Average AUDPC, average of the area under disease curve progress; Average BCI, average of the biological control index; P1, compost P1; P1t, compost P1 with thermal treatment; P2, compost P2; P2t, compost P2 with thermal treatment, P, peat; Pt, peat with thermal treatment. Bars depicting the same character have no statistically significant differences for $p<0,05$ (Duncan test).

675 Table 1. Properties of composts, at the end of the composting process, and peat.

	pH	**CE ($dS.m^{-1}$)**	**OM (%)**	**N (%)**	**C/N**	**DM (%)**	**Zucconi índex (%)**
P1	7.06b***	1.76b*	68.6c***	1.99a*	20.1b***	50.3a	85.2***
P2	7.33a***	2.67a*	81.1b***	1.68b*	28.9a***	46.5b	91.8***
T	6.00c	0.126c	93.4a	2.11a	50.5a	22.8c	

676 P1, compost P1; P2, compost P2; P, peat; CE, electrical conductivity; OM, organic
677 matter; N, nitrogen; C/N, C/N ratio; DM, dry matter. To compare organic compost,
678 according to Student T test, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$,. For all three substrate
679 values with the same letter had not statistical differences ($p<0,05$), according to Duncan
680 New Multiple-Range Test.

681

682 Table 2. Microbial populations (CFUs g^{-1} compost) at the end of the composting process
683 and number of biological control agents isolated in the different phases (CP, MP and
684 FP) of the composting process.

C	Bacteria	Fungi	Actinomycetes	Chitin.	Celulol.	Biological control agents		
						CP	MP	FP
P1	$6.00x10^{9}$*	$7.58x10^{10}$*	$2.40x10^{7}$	$1.9x10^{6}$**	$3.14x10^{5}$*	2	3	9
P2	$2.02x10^{11}$*	$5.26x10^{11}$*	$6.86x10^{7}$	$1.6x10^{7}$**	$7.83x10^{5}$*	2	5	10

685 C, compost; P1, compost P1; P2, compost P2; Chitin., chitinolytic microorganisms;
686 Celulol., cellulolytic microorganisms; CP, Cooling phase (temperature < 45 ºC); MP,
687 Maturation phase (temperature < 30 ºC); FP, Final product (room temperature);
688 *$p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$, according to Student T test.

689

690

691 Table 3. Inhibition percentage of the pathogenic fungi by potential antagonistics, by
692 each compost (P1 and P2).

P1 – Pathogenic fungi inhibition (%)			
Isolates strain - Lab. Code	*Clarireedia* spp.	*S. rolfsii*	*R. solani*
T. atroviride – A	59.5 bcd	58.5 abc	60.2 ab
Purpureocillium sp. - A	29.8 j	25.8 de	49.8 efgh
Purpureocillium sp. - B	45.9 gh	35.3 bcde	40.5 i
F. keratoplasticum - A	62.4 abc	49.5 abcde	39.7 i
F. oxysporum – A	30.5 j	35.1 bcde	50.8 defgh
F. oxysporum - B	44.5 gh	35.9 bcde	48.8 fgh
Penicillium sp. - A	41.9 hi	34.1 cde	40.3 i
F. keratoplasticum - B	48.2 fg	35.8 bcde	45.6 ghi
T. atroviride – B	64.2 ab	63.6 a	58.7 abcd
T. atroviride – C	65.0 ab	58.9 abc	62.5 a
Purpureocillium sp. - C	29.8 j	25.8 de	49.8 efgh
Purpureocillium sp. - D	45.9 gh	35.3 de	40.6i
F. lichenicola - A	45.2 gh	35.2 bcde	49.1 fgh
M. anisopliae - A	30.2 j	35.4 de	27.5 bcdefg

P2 – Pathogenic fungi inhibition (%)			
	Clarireedia spp.	*S. rolfsii*	*R. solani*
T. atroviride – D	63.4ab	63.8 a	56.4 abcdef
T. atroviride – E	66.3a	60.6 ab	57.5 abcde
T. atroviride – F	66.7a	63.6 a	58.6 abcd
Beauveria sp. - A	37.3 i	31.4 de	46.4 ghi
T. atroviride – G	64.2 ab	58.9 abc	62.5 a

T. asperellum - A	57.6 cd	57.1 abcd	61.6 a
Penicillium sp. - B	26.9 j	41.6 abcde	42.8 hi
T. atroviride – H	67.5 a	62.8 a	59.4 abc
Purpureocillium sp. - E	37.7 i	33.5 cde	27.4 bcdefg
B. ochroleuca – A	52.4 ef	34.4 bcde	52.4 cdefg
B. ochroleuca – B	54.6 de	34.4 bcde	51.5 hi
F. oxysporum - C	38.0 i	36.1 bcde	42.5 hi
F. oxysporum – C	47.5 fg	57.7 sbcd	45.9 ghi
B. ochroleuca - C	54.6 de	34.4 bcde	51.5 cdefg
F. lichenicola - B	45.7 gh	35.4 bcde	49.3 fgh
M. anisopliae - B	30.5 j	35.9 bcde	27.6 bcdefg
C. rose - A	42.3 hi	42.1 abcde	43.1 hi

693 *R. solani, Rhizoctinia solani; S. rolfsii, Sclerotium rolfsii*; P1, compost P1; P2, compost
694 P2. For each disease, values with the same letter had not statistical differences ($p<0.05$),
695 according to Duncan New Multiple-Range Test. Capital letter after the species name
696 corresponds to the Lab. Code for the isolated strain.
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Fig. 1. Compost effectiveness in the biological control of the *Rhizoctinia solani*. Average area, average of the affected area by disease; Average Severity, average of the disease severity; Average AUDPC, average of the area under disease curve progress; Average BCI, average of the biological control index; P1, compost P1; P1t, compost P1 with thermal treatment; P2, compost P2; P2t, compost P2 with thermal treatment, P, peat; Pt, peat with thermal treatment. Bars depicting the same character have no statistically significant differences for $p<0,05$ (Duncan test).

Fig. 2. Compost effectiveness in the biological control of the *Clarireedia spp.*. Average area, average of the affected area by disease; Average Severity, average of the disease severity; Average AUDPC, average of the area under disease curve progress; Average BCI, average of the biological control index; P1, compost P1; P1t, compost P1 with thermal treatment; P2, compost P2; P2t, compost P2 with thermal treatment, P, peat; Pt, peat with thermal treatment. Bars depicting the same character have no statistically significant differences for $p<0,05$ (Duncan test).

Fig. 3. Compost effectiveness in the biological control of the *Sclerotium rolfsii*. Average area, average of the affected area by disease; Average Severity, average of the disease severity; Average AUDPC, average of the area under disease curve progress; Average BCI, average of the biological control index; P1, compost P1; P1t, compost P1 with thermal treatment; P2, compost P2; P2t, compost P2 with thermal treatment, P, peat; Pt, peat with thermal treatment. Bars depicting the same character have no statistically significant differences for $p<0,05$ (Duncan test).

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748 P2. For each disease, values with the same letter had not statistical differences ($p<0.05$).
749 Capital letter after the species name corresponds to the Lab. Code for the isolated strain.