Antagonistic potential of certain soilborne fungal bioagents against *Monosporascus* root rot and vine decline of watermelon and promotion of its growth

Abdelhak Rhouma¹, Ibtissem Ben Salem¹, Mahmoud M’Hamdi², Naima Boughalleb-M’Hamdi¹*

¹Département des Sciences Biologiques et de la Protection des Plantes, Institut Supérieur Agronomique de Chott Mariem, 4042 Sousse, UR13AGR03, Université de Sousse, Tunisie; ²Département des Sciences Horticoles et du Paysage, 4042 Sousse, Institut Supérieur Agronomique de Chott Mariem, 4042 Sousse, Université de Sousse, Tunisie.

*Correspondence author E-mail: n.boughalleb2017@gmail.com

Received: 9 October, 2018; Accepted: 22 October, 2018; Published online: 29 October, 2018

Abstract

*Monosporascus cannonballus* responsible for cucurbits *Monosporascus* root rot and vine decline, is worldwide spread notably in Tunisia. The most appropriate strategies to suppress disease development are those able to reduce the ascospores population using eco-friendly approach treatments. Seven soilborne fungal isolates were tested *in vitro* (by dual confrontation technique) and *in vivo* in the greenhouse as potential bioagents against three virulent *M. cannonballus* isolates. *In vivo* experiments were divided into two assays, preventive and curative treatments. *Trichoderma viride* and *T. harzianum* exhibited high inhibitory activities against *M. cannonballus* mycelial growth with values more than 90%, followed by *Aspergillus niger* (87.89%) and *Paecilomyces victoriae* (80.44%). Furthermore, these two *Trichoderma* spp. when applied preventively and curatively in *in vivo* trials, reduced significantly disease incidence (8.33% and 16.67-20.83%), root disease index (0.79-0.8 and 1.25-1.17), and reduced also ascospores index (1.5-1.54 asc/g of peat) and (2.54-2.42 asc/g of peat), respectively, in comparison with control treatments. Moreover, *T. viride* and *T. harzianum* enhanced the growth development of watermelon plants treated preventively and curatively in the greenhouse. They significantly improved different horticultural measurements with mean values of plant height (76.75-79.83 cm, and 81.83-80.92 cm), root volume (2.39-2.22 cm³, and 1.84-1.88 cm³), above grounds fresh weight (16.07-16.57 g, and 12.84-14.93 g) and dry wt. (2.49-2.6 g, and 2.66-2.70 g), underground fresh wt. (0.725-0.654 g, and 0.717-0.690 g) and dry wt. (0.147-0.214 g, and 0.156-0.152 g). Based on current results, it appears that *Trichoderma* spp. could be employed in soil treatments to promote watermelon plant growth and development.

**Keywords:** *Monosporascus cannonballus*, Ascospores population, Biocontrol activity, *Trichoderma* spp., *in vitro* antagonism, Horticultural measurement
1. Introduction

Several fungal species cause worldwide plant lesions, rots, loss of secondary; tertiary and feeder roots. These are associated with sudden and uniform collapse of entire fields 1-2 weeks prior to harvest, resulting in total crop loss. The main species associated with these syndromes is *M. cannonballus* (Cohen et al., 2000). Indeed, the onset of root infection occurs during early stages of growth, followed by wilting and death of plants later in the season (Cohen et al., 2012). *Monosporascus* root rot and vine decline (MRRVD) is particularly severe in arid and semi-arid worldwide cucurbit production. According to Boughalleb et al., (2010); Ben Salem et al., (2013); Rhouma et al., (2018), this disease is prominent in several melon and watermelon-producing areas in Tunisia, and can infect and produce perithecia in different cucurbit roots (Mertely et al., 1993).

Investigations on the biology of *M. cannonballus* demonstrated that its ascospores function as the only known survival structures in soil (Stanghellini et al., 2000). Furthermore, Waugh et al., (2003) pointed that one melon plant infected by *M. cannonballus* could support the production of approximately 400,000 ascospores. Theses authors added that fields are considered problematic when the soil is infected with two ascospores/ g of soil which could be associated with significant crop losses, and concluded that *M. cannonballus* is a monocyclic pathogen. Consequently, this pathogen has a great potential to maintain and/or increase its inoculum build up in the cucurbit rhizosphere (Cohen et al., 2012). Waugh et al., (2003) reported that management of *M. cannonballus* can be accomplished in case of early detection and quantification of its primary inoculum. In several studies, ascospores were extracted from soils through a physical method based on a sucrose centrifugation technique (Stanghellini and Rasmussen, 1992; Boughalleb et al., 2010).

The most appropriate strategies used to suppress plant disease development were those able to reduce the size of pathogen population (Fry, 1982). Control of MRRVD is currently based on integrating different approaches (Cohen et al., 2012; Ben Salem et al., 2015a). Farmers used to apply fungicides treatments (Cohen et al., 2007; Ben Salem et al., 2015b); however, these chemical methods cause hazards to human health and increase environmental pollution. Therefore, alternatives methods are required for plant diseases control. Biological control is the best alternative and eco-friendly approach for such treatments, defined as total or partial destruction of pathogen populations by other organisms, which occur routinely in nature (Rojo et al., 2007). For example, the use of *Trichoderma* spp. (Menatoullah et al., 2010; Boughalleb-M’Hamdi et al., 2018), and *Chaetomium* spp. (Sales et al., 2007) against MRRVD presented high efficacy when tested under *in vitro* and *in vivo* conditions. Reda et al., (2008) revealed that beneficial bacteria are also able to inhibit *M. cannonballus* growth and induce resistance in melon. The objective of the present investigation was to screen certain soilborne fungal antagonist’s for abilities to reduce *M. cannonballus* growth under *in vitro* and *in vivo* conditions.

2. Materials and methods

2.1. Fungal cultures

Seven antagonistic fungal isolates namely; *T. viride*, *T. harzianum*, *Penicillium purpurascens*, *Chaetomium globosum*, *Aspergillus niger*, *A. glaucus* and *Paecilomyces victoria*, were isolated from Tunisian cucurbit rhizosphere from a field located at Chott Meriem, (Sousse). Meanwhile, three highly virulent isolates of *M. cannonballus* (MT3, MT4 and MT41) were recovered from cucurbit plant in experimental field of the High Institute of Agronomy, Chott Meriem, Tunisia.
2.2. In vitro antifungal potential of fungal bioagents against pathogenic M. cannonballus

Antifungal activities of the seven fungal antagonists on radial mycelial growth of the three pathogenic M. cannonballus isolates was determined by dual confrontation technique on Potato dextrose agar (PDA) according to Boughalleb-M’Hamdi et al., (2017).

Two discs plugs (0.5 cm diameter) of each pathogen and antagonist (4 days-old culture) were transferred separately to a single PDA plate (9 cm diameter). The antagonist plug was placed on one side of the plate (about 2 cm from the edge of the plate towards the center), while the pathogen plug was placed at the other side of the plate opposite to the antagonist plug, leaving a distance of 5 cm between the two plugs. A plug of PDA medium was used as control treatment, while the pathogen plug was placed at the other side. Three replicates (two plates / replicate) for each individual treatment were conducted and the plates were incubated at 28 ± 2°C for five days. The percent of inhibition of pathogen radial mycelial growth was evaluated according to the formula of Hmouni et al., (1996):

\[ I(\%) = \left(1 - \frac{C_n}{C_0}\right) \times 100 \]

Where: Cn is the diameter of radial growth of the pathogen in the presence of the antagonist, whereas, C0 is the diameter of growth of the pathogen in the control treatment.

2.3. In vivo antifungal potential of the fungal bioagents

In vivo experiments were divided into two assays of preventive and curative treatments at March, 2015 in the greenhouse. The first preventive assay was carried out by dipping roots of watermelon seedlings (cv. Crimson sweet) 15 days old, into a flask containing a conidial suspension of the different antagonists (3×10^8 cfu/ml each) for 30 min. 24 h before adding 50 ml (9×10^8 cfu/ml) of each pathogenic isolate, separately. For curative treatments, watermelon seedlings were treated with each antagonist separately 7 days after inoculation of each pathogen, by adding 10 ml of fungal antagonist’s suspension to each pot (3×10^8 cfu/ml). Watermelon seeds were sown in nursery seed trays, with 18 plants per each treatment having 3 replicates. The soil substrate used in this in vivo experiment consisted of a mixture of peat and vermiculite (1:1), which was autoclaved twice at 120°C. The pots were then placed in a greenhouse for 60 days. Two controls were performed; one by inoculating the plants with the pathogen only (positive control), while the other with dist. water (negative control). The experimental design was a randomized complete block design (RCBD), and the entire experiment was repeated twice.

Inoculation of the soil substrate with M. cannonballus ascospores only was performed as reported by Stanghellini et al., (2000); Aleandri et al., (2017), with some modifications. M. cannonballus isolates were obtained from two-month-old PDA agar cultures, perithecia were washed and then ascospores were sieved (32 µm). Ascospores concentration was adjusted with dist. water (5 ascospores/g of peat). All growth parameters were measured 2 months after inoculation.

The number of symptomatic plants and the total number of plants evaluated in each treatment were used to estimate the disease incidence (DI) of MRRVD, by using the following formula: DI (%) = (Total no. of symptomatic plants/ Total no. of plants) x 100 in reference to Ben Salem et al., (2015a).

Watermelon plants were carefully removed after 2 months, the root system was then gently washed in tap water. Roots were inspected visually for evidence of root necrosis, and for observing roots bearing perithecia of M. cannonballus containing single spored asci. Each root system was rated for the severity of M. cannonballus lesions using a root disease index (RDI) which is an adapted scale from...
Aegerter et al., (2000), where 0 = no symptoms; 1 = few lesions (covering <10% of root) and secondary root rot is slight; 2 = rot of secondary roots or lesions covering approximately 25% of the root; 3 = lesions covering at least 50% of the root and dead secondary roots; and 4 = general root rot where most of the root is affected.

Soil samples treated with seven fungal antagonists and inoculated with three *M. cannonballus* isolates separately, were air-dried at room temperature and sieved through a 2-mm mesh before their ascospores quantification was accomplished. *M. cannonballus* ascospores were extracted by a method adopted from Boughalleb et al., (2010). Initially, sub-samples were sieved through a 250 µm sieve. A 20-g subsample was placed in 200 ml of water, agitated for 5 min. and then passed through two superposed sieves (75 and 30 µm). The collected material was washed and centrifuged at 2000 g for 4 min. The supernatant was discarded and then 30-40 ml of 50% sucrose solution was added to the pellet and then centrifuged again for 2 min. at 2000 g. After centrifugation, the supernatant was passed through a mesh of 30 µm. The materials retained were distributed in Petri dishes. This suspension was stored at 4°C until being analyzed. The ascospores characteristics and count were done under a stereomicroscope (Nikon SMZ 1000) at a magnification of x60. After the initial (Pi = 5 asc/g peat) and final (Pf) ascospores count, the following formula was applied to determine the percentage of the ascospores index (AI) according to Ferreira, (2011):

\[
AI (\%) = (1-Pi/Pf) \times 100
\]

After determination of the fresh wt. of above ground (stem + leaves) and underground (root) portions, plant samples were placed in an oven at 60°C for 48 h to determine the dry wt. (Heitholt, 1989). The height of the plant was measured (cm) using a flat ruler. Root volume (cm³) was determined by the immersion method as described by Musick et al., (1965), through comparing the levels of water before and after immersing the whole root in a known volume of this water.

### 2.4. Statistical analysis

Data were analyzed by ANOVA using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA). Differences between treatments were determined by Duncan multiple range test at 5% of significance level.

### 3. Results

#### 3.1. In vitro antifungal efficacy of bioagents against *M. cannonballus* isolates on PDA

The seven antagonistic fungal isolates exerted high significant reduction (<0.01) on radial mycelial growth of *M. cannonballus* isolates after five days of incubation. The linear decrease of growth of all the pathogenic isolates ranged from 95.16% (MT41/*T. harzianum*) to 47.25% (MT3/*P. purpurascens*) (Table 1). Statistical analysis revealed high significant interactions between *M. cannonballus* isolates and the antagonists (<0.01).

The two *Trichoderma* spp. showed a good ability to limit the mycelial growth of all *M. cannonballus* isolates in vitro. In fact, the mycelial growth of the three *M. cannonballus* isolates decreased in presence of *T. viride* and *T. harzianum* with values ranging between 91.93 and 92.11%, respectively (Table 1, Fig. 1). Moreover, *in vitro* assay revealed that *A. niger* possessed a good antifungal potency with mycelial inhibition rate between 88.7% (MT3) and 87.89% (MT4), followed by *Paecilomyces victoriae* (80.44%) and *P. purpurascens* (48.15%) (Table 1). This antagonistic potency was not only on the mycelial growth reduction, but also on the microscopic hyphal aspect. Compared to controls, *M. cannonballus* isolates treated with *Trichoderma* spp. and *A. niger* exhibited a mycelium with strong lyses, induction of mycelial cords via anastomosis between hyphal filaments and mycelium winding (Fig. 1).
Fig. 1: A: Dual confrontation of *M. cannonballus* after incubation at 28°C for five days on PDA. a) Control. b) *P. purpurascens*. c) *A. glaucus*. d) *C. globosum*. e) *A. niger*. f) *T. harzianum*. g) *T. viride*. h) *Paecilomyces victoriae*; B: *In vitro* mycelial interaction between *M. cannonballus* and antagonists (*T. viride*, *T. harzianum* and *A. niger*) (a, b, c, d and e) after 5 days of incubation at 28°C on PDA medium (Gr x 40) (one plate per treatment was illustrated), revealing (a) lyses of pathogen fungal mycelia in the presence of *T. viride*, (c) *T. harzianum* and (d) *A. niger*; (b) transformation into cords and (e) mycelium rolling up.

**Table 1**: Effect of direct confrontation of seven fungal antagonists on mycelial growth inhibition of three *M. cannonballus* isolates (MT3, MT4 and MT41) after five days of incubation at 28°C

| Antagonists spp. | Mycelial growth inhibition percentage (%) | P-value<sup>d</sup> |
|------------------|------------------------------------------|---------------------|
|                  | MT3            | MT4            | MT41          | Mean    |                   |
| *T. viride*      | 89.23±0.20a<sup>C</sup> | 91.93±0.24aB    | 94.62±0.19aA  | 91.93    | <0.01             |
| *T. harzianum*   | 89.23±0.29aB   | 91.93±0.40aB    | 95.16±0.40aA  | 92.11    | <0.01             |
| *P. purpurascens*| 47.25±0.46eB   | 48.33±0.24eAB   | 48.86±0.21fA  | 48.15    | <0.05             |
| *C. globosum*    | 56.94±0.37dB   | 65.82±0.46dA    | 51.02±0.30eC  | 57.93    | <0.01             |
| *A. niger*       | 88.7±0.30a     | 87.89±0.40b     | 87.08±0.23b   | 87.89    | ≥0.05             |
| *A. glaucus*     | 75.24±0.28eA   | 68.78±0.23dB    | 69.32±0.17dB  | 71.11    | <0.01             |
| *Paecilomyces victoriae* | 80.08±0.29bB | 82.78±0.25cA | 78.47±0.25cB | 80.44    | <0.01             |
| **Mean**         | 75.24          | 76.78          | 74.93         | nd       | nd                 |

<sup>a</sup> Inhibition percent of mycelial growth formula: I (%) = (1 - Cn/Co) x 100 where Cn is the mean diameter of the colonies in the presence of the antagonist, and Co the mean diameter of the control colonies, means of nine Petri dishes (three plates per replicate). Duncan’s Multiple Range Test, values followed by different letters are significantly different at P≤0.05. <sup>b</sup>Duncan’s Multiple Range Test is for comparison of mycelial growth inhibition means among fungal antagonists for the same isolate. Small letters are for means of comparison in the same row. <sup>c</sup>Duncan’s Multiple Range Test is for comparison of mycelial growth inhibition means among isolates for the same fungal antagonist. Capital letters are for means comparison in the same column. <sup>d</sup>Probabilities associated with individual F tests. nd: not determined.
3.2. *In vivo* antifungal potential of the fungal bioagents on watermelon plants infested with *M. cannonballus* in the greenhouse

Statistical analysis indicated that plants inoculated with *M. cannonballus* isolates and treated preventively and curatively by the seven antagonistic fungi was highly significant (<0.01). However, no difference was detected between *M. cannonballus* isolates (≥0.05). Watermelon plants seemed healthy with no symptoms of *M. cannonballus* infection (disease incidence = 0%), when treated with *T. viride*, *T. harzianum*, *C. globosum* and *A. glaucus* for MT3 isolate; *T. viride*, *T. harzianum*, *P. purpurascens* and *A. niger* for MT4, and *P. purpurascens*, *C. globosum* and *A. niger* for MT41 isolate, when used as preventive treatment (positive control = 100%; negative control = 0%). However, when plants were treated curatively, the antagonists showed varied antifungal activity with mean value of disease incidence 34.72% (ranging between 0 (MT4/ *Paecilomyces victoriae*) and 100% (MT4/ *A. glaucus*), and 40.28% (ranging between 0 (MT3/ *T. viride*) and 75% (MT3/ *Paecilomyces victoriae*; MT3/ *P. purpurascens*)). These obtained results indicated that *T. viride* and *T. harzianum* applied curatively reduced significantly disease incidence, recording the lowest value of 25% compared with positive control (100%) and negative control (0%) (Table 2). These findings were confirmed after 2 months by above ground symptoms on watermelon plants infested with *M. cannonballus* isolates (Fig. 2).

Fig. 2: Above-ground symptoms observed on watermelon plants inoculated with three *M. cannonballus* isolates and treated preventively and curatively by seven fungal antagonists after 2 months in the greenhouse. a: watermelon treated preventively by *T. harzianum* (no collapse); b: watermelon treated preventively by *T. viride* (no collapse); c: negative control (no collapse); d: watermelon treated curatively by *T. harzianum* (initial wilting and reversible turgor loss); e: watermelon treated curatively by *T. viride* (initial wilting and reversible turgor loss); f: positive control (total collapse of all plants).
Table 2: Disease incidence of Monosporascus root rot and vine decline (MRRVD) recorded by watermelon seedlings inoculated with three M. cannonballus isolates (MT3, MT4 and MT41) and treated preventively and curatively with seven antagonist’s *in vivo* assay. Positive and negative controls were performed by inoculating the plant with the pathogen only, and with dist. water, respectively.

| Treatments                  | MT3         | MT4         | MT41        | Mean  | P-value<sup>b</sup> |
|-----------------------------|-------------|-------------|-------------|-------|--------------------|
| **Preventive treatments**   |             |             |             |       |                    |
| Positive control            | 100±0a<sup>a</sup> | 100±0a     | 100±0a     | 100   | nd                 |
| Negative control            | 0±0b        | 0±0c        | 0±0b        | 0     | nd                 |
| T. viride                   | 0±0b        | 0±0c        | 25±1.77b    | 8.33  | ≥0.05              |
| T. harzianum                | 0±0b        | 0±0c        | 25±1.77b    | 8.33  | ≥0.05              |
| P. purpurascens             | 25±1.77b    | 0±0c        | 0±0b        | 8.33  | ≥0.05              |
| C. globosum                 | 0±0b        | 25±1.77bc   | 0±0b        | 8.33  | ≥0.05              |
| A. niger                    | 12.5±1.25b  | 0±0c        | 0±0b        | 4.17  | ≥0.05              |
| A. glaucus                  | 0±0b        | 25±1.34bc   | 25±1.77b    | 16.67 | ≥0.05              |
| Paecilomyces victoriae      | 12.5±1.25b  | 37.5±1.25b  | 0±0b        | 16.67 | ≥0.05              |
| Mean                        | 16.67       | 20.83       | 19.44       |       |                    |
| P-value<sup>a</sup>         | <0.01       | <0.01       | <0.01       | nd    | nd                 |

| Treatments                  | MT3         | MT4         | MT41        | Mean  | P-value<sup>b</sup> |
|-----------------------------|-------------|-------------|-------------|-------|--------------------|
| **Curative treatments**     |             |             |             |       |                    |
| Positive control            | 100±0a<sup>a</sup> | 100±0a     | 100±0a     | 100   | nd                 |
| Negative control            | 0±0c        | 0±0c        | 0±0b        | 0     | nd                 |
| T. viride                   | 0±0c        | 25±1.77bc   | 25±1.77ab   | 16.67 | ≥0.05              |
| T. harzianum                | 12.5±1.25bc | 25±1.77bc   | 25±1.77ab   | 20.83 | ≥0.05              |
| P. purpurascens             | 75±1.77ab   | 75±1.77ab   | 37.5±1.73ab | 62.50 | ≥0.05              |
| C. globosum                 | 25±1.77bc   | 50±1.9ab    | 50±1.9ab    | 41.67 | ≥0.05              |
| A. niger                    | 25±1.77bc   | 37.5±1.73bc | 50±1.9ab    | 37.50 | ≥0.05              |
| A. glaucus                  | 50±1.9abc   | 100±0a      | 75±1.77ab   | 75    | ≥0.05              |
| Paecilomyces victoriae      | 75±1.77ab   | 0±0c        | 75±1.9ab    | 50    | ≥0.05              |
| Mean                        | 40.28       | 34.72       | 37.50       | nd    | nd                 |
| P-value<sup>a</sup>         | <0.01       | <0.01       | <0.01       | nd    | nd                 |

<sup>a</sup> Duncan’s Multiple Range Test is for disease incidence of MRRVD mean values showing comparison among the three *M. cannonballus* isolates (Means of 6 plants per each of three replicates). <sup>b</sup> Probabilities associated with individual F tests. nd: not determined. The disease incidence of MRRVD was calculated by using the following formula: DI (%) = (Total no. of symptomatic plants/ Total .o. of plants) x 100.

Watermelon plants treated preventively with *P. purpurascens*, *C. globosum*, *A. niger* and *A. glaucus* showed disease symptoms on roots with relatively high disease severity index values of 1.55, 2.04, 1.88 and 1.84, respectively. However, the lowest value was noted on plants treated with *T. viride* (0.79) and *T. harzianum* (0.80) (positive control = 3.71; negative control = 0) (Table 3). Perithecia of *M. cannonballus* were not observed on watermelon roots treated preventively with *T. viride*, *T. harzianum* and *A. niger*.

Observing the infested roots treated curatively, *T. viride* and *T. harzianum* showed the most significant reduction of disease severity index with values ranged between 1.25 (0.63 (MT4) -2 (MT41)), and 1.17 (0.88 (MT3) -1.5 (MT41)), respectively, whereas, positive control value = 3.71 (Table 3). In addition, perithecia of this pathogen were not observed on roots treated curatively by these two *Trichoderma* spp. However, *M. cannonballus* isolates were pathogenic on watermelon plants in the presence of some antagonists such as; *C. globosum*, *A. niger*, *A. glaucus* and *P. victoriae* with root severity mean values of 2.33, 2.54, 2.17 and 2.42, respectively. These infested watermelon plants showed roots with typical symptoms of MRRVD including; lesions, rots, loss of secondary, tertiary and feeder roots, in...
addition to production of perithecia (Fig. 3).

![Image](image_url)

**Fig. 3**: Roots inoculated with three *M. cannonballus* isolates and treated preventively and curatively with seven antagonist’s showing *in vivo* symptoms in the greenhouse. a: *M. cannonballus* alone (positive control); b: uninoculated plants (negative control); c: *T. viride* + *M. cannonballus* after a preventive treatment; d: *T. viride* + *M. cannonballus* after a curative treatment; e: *T. harzianum* + *M. cannonballus* after a curative treatment; f: *T. harzianum* + *M. cannonballus* after a preventive treatment.

**Table 3**: Root disease index recorded by watermelon seedlings inoculated with three *M. cannonballus* isolates (MT3, MT4 and MT41) and treated preventively and curatively with seven antagonistic fungal species in the greenhouse. Positive and negative controls were performed by inoculating the plants only with the pathogen and with dist. water, respectively.

| Treatments | Preventive treatments | Curative treatments |
|------------|-----------------------|---------------------|
|            | MT3                   | MT4 | MT41 | Mean | P-value |
| Positive control | 3.58±0.25a^a^ | 3.54±0.29a | 3.24±0.58a | 3.45 | ≥0.05 |
| Negative control | 0±0e | 0±0f | 0±0e | 0 | nd |
| *T. viride* | 0.75±0.29d | 0.75±0.5e | 0.88±0.25d | 0.79 | ≥0.05 |
| *T. harzianum* | 0.63±0.25d | 0.88±0.25e | 0.88±0.48d | 0.80 | ≥0.05 |
| *P. purpurascens* | 1.88±0.48b | 1.38±0.48de | 1.38±0.48cd | 1.55 | ≥0.05 |
| *C. globosum* | 1.5±0.71bc | 2.5±0.41b | 2.13±0.75b | 2.04 | ≥0.05 |
| *A. niger* | 1.25±0.65bcd | 2.25±0.65bc | 2.13±0.63b | 1.88 | ≥0.05 |
| *A. glaucus* | 1.88±0.25b | 1.75±0.25cd | 1.88±0.25bc | 1.84 | ≥0.05 |
| *Paecilomyces victoriae* | 1±0.41cd | 1.38±0.25de | 1.25±0.29cd | 1.21 | ≥0.05 |
| Mean | 1.39 | 1.61 | 1.53 | nd | nd |
| P-value | <0.01 | <0.01 | <0.01 | nd | nd |
3.3. In vivo potency of fungal biocontrol agents on ascospores populations of *M. cannonballus*

Application of antagonists preventively and curatively reduced the ascospores population levels (<0.01) (Tables 4 and 5). Results revealed and confirmed the efficiency of both *T. harzianum* and *T. viride* isolates by decreasing significantly the ascospores densities which varied from 1.42 (MT41) - 1.69 (MT3) asc/g of peat, and between 1.29 (MT41) - 1.73 (MT3) asc/g peat, respectively (positive control = 5.5 asc/g of peat). The lowest reduction of ascospores number was registered on plants treated by *T. harzianum* and *T. viride* with means of -260.68, and -243.47%, respectively. The effect of the other antagonists varied between 3.28 (*Paecilomyces victoriae*) - 4.23 (*P. purpurascens*) asc/g peat. The decrease of ascospores index ranged from -52.54 to -18.17%, respectively (positive control = 8.5%).

When plants were curatively treated with *T. harzianum* and *T. viride*, they reduced significantly the ascospores population with values of 2.42 (IA= -109.55) asc/g of peat, and 2.45 (IA= -110.24) asc/g of peat, respectively. However, the other antagonists expressed less efficiency with respect to ascospores densities varying between 3.76 (*P. purpurascens*) asc/g of peat - 4.61 (*Paecilomyces victoriae*) asc/g of peat. The reduction percent of *M. cannonballus* ascospores ranged from -33.3 and -8.46% for *P. purpurascens* and *Paecilomyces victoriae* respectively (positive control = 5.42 asc/g of peat; IA of positive control = 7.25%) Tables (4 and 5).

3.4. In vivo potency of fungal biocontrol agents in promoting growth parameters of watermelon plants infested with *M. cannonballus*

The interaction between *M. cannonballus* and the seven antagonists was significant (p<0.05). However, there were no significant differences between *M. cannonballus* isolates. Growth promotion results for watermelon plants treated preventively are presented in (Tables 4 and 5). All treatments differed significantly from positive control. The best treatment was the combination of *T. harzianum* and *T. viride* with *M. cannonballus* isolate (MT4), which showed significant increase in growth parameters of aboveground parts, compared with the other treatments. The values of the aboveground fresh wt. were about 18.138 and 16.5 g, plant height were of 79 and 83 cm, however, aboveground dry wt. with MT3 were (2.65 and 6.52 g). After 9 weeks in the greenhouse, the root system was collected from all treatments and checked. Both MT3 and MT4 isolates produced fewer roots of infested plants. The underground fresh, dry wt. and root volume values for plants treated with *T. viride* ranged between 0.688 g (MT41) and 0.758 g (MT3), from 0.138 g (MT3) to 0.125 g (MT4 and MT41), and from 2.025 cm3 (MT3) to 2.825 cm3 (MT41), for the three growth parameters, respectively. For *T. harzianum*, results revealed low difference compared with the previous values. The improvement rates of the three growth parameters compared with the negative and positive controls were 98% - 260% for underground fresh wt., 87.5 % - 400% for underground dry wt., and 102% - 400 % for root volume, respectively. Watermelon plants treated curatively showed an increase of the above and underground growth parameters compared with the positive control, however, there were a slight difference for plants treated preventively.
Table (4): Ascospores population dynamics recorded by watermelon seedlings inoculated with three *M. cannonballus* isolates (MT3, MT4 and MT41), and treated preventively and curatively with seven fungal biocontrol agents in vivo assay. Positive control was inoculated with the pathogen only.

| Treatments                  | Preventive treatments | Curative treatments | P-value |
|-----------------------------|-----------------------|---------------------|---------|
|                             | MT3       | MT4       | MT41     | Mean |          | MT3       | MT4       | MT41     |
| **Positive control**        | 5.29±0.23a | 5.96±0.51a | 5.25±0.6a | 5.5  | ≥0.05    | 5.41±0.23a | 5.66±0.51a | 5.20±0.53a | 5.42 | ≥0.05 |
| *T. viride*                 | 1.73±0.06eA  | 1.48±0.32dAB | 1.29±0.06eB | 1.5  | <0.05    | 2.58±0.23e  | 2.66±0.25e  | 2.14±0.46c  | 2.45 | ≥0.05 |
| *T. harzianum*              | 1.69±0.28e  | 1.5±0.61d   | 1.42±0.29e  | 1.54 | ≥0.05    | 2.7±0.14eA  | 2.26±0.19fB | 2.29±0.26cB | 2.42 | <0.05 |
| *P. purpurascens*           | 4.23±0.09b  | 4.26±0.08b  | 4.21±0.14b  | 4.23 | ≥0.05    | 3.71±0.11d  | 3.83±0.1d   | 3.73±0.13b  | 3.76 | ≥0.05 |
| *C. globosum*               | 3.83±0.1c   | 3.79±0.13bc  | 3.76±0.13c  | 3.79 | ≥0.05    | 4.3±0.13c   | 4.35±0.37bc  | 4.09±0.68b  | 4.25 | ≥0.05 |
| *A. niger*                  | 3.79±0.21c  | 3.79±0.45bc  | 3.68±0.13c  | 3.75 | ≥0.05    | 4.08±0.02c  | 4.16±0.05cd  | 4.26±0.06b  | 4.17 | ≥0.05 |
| *A. glaucus*                | 3.78±0.13c  | 3.75±0.09bc  | 3.71±0.19c  | 3.75 | ≥0.05    | 4.28±0.09c  | 4.29±0.05c   | 3.83±0.95b  | 4.13 | ≥0.05 |
| *Paecilomyces victoriae*    | 3.21±0.13dB  | 3.43±0.03A   | 3.21±0.13dB | 3.28 | <0.05    | 4.65±0.11bA  | 4.69±0.09bA  | 4.52±0.07abB | 4.61 | <0.05 |
| **Mean**                    | 3.44       | 3.5        | 3.32      | nd   | nd       | 3.96       | 3.99       | 3.75      | nd   | nd     |
| **P-value**                 | <0.01      | <0.01      | <0.01     | nd   | nd       | <0.01      | <0.01      | <0.01     | nd   | nd     |

a Duncan’s Multiple Range Test for ascospores population means in comparison among the three *M. cannonballus* isolates treated with different antagonistic fungal spp.; Small letters are for means of comparison of the different antagonists in the same column. b Duncan’s Multiple Range Test is for ascospores population means in comparison among the seven antagonistic fungal spp. for the different *M. cannonballus* isolates; Capital letters are for comparison of means in the same row (Means of 6 plants per each of three replicates). c Probabilities associated with individual F tests. nd: not determined.

Indeed, *T. harzianum* presented a good improvement of the above (14.93 g) and underground (0.717 g) fresh wt., and above and underground dry wt. (2.7 and 0.156 g, respectively). *Trichoderma* treatments were very effective against *M. cannonballus* infested watermelon plants; the severity of infection was reduced and the growth parameters of these plants improved as well.

4. Discussion

The control of soilborne pathogens was difficult as they produce viable structures such as ascospores which were resistant to adverse environmental conditions (Cohen *et al*., 2000). However, Rojo *et al*., (2007) pointed that the misuse of fungicides to manage these pathogens caused enormous problems to ecosystem and human’s health.
Table 5: Percentage of *M. cannonballus* ascospores index recorded by watermelon seedlings inoculated with three *M. cannonballus* isolates (MT3, MT4 and MT41), and treated preventively and curatively with seven fungal biocontrol agents *in vivo* assay. Positive controls were inoculated with the pathogen only.

| Treatments                  | MT3        | MT4        | MT41       | Mean | P-value* |
|-----------------------------|------------|------------|------------|------|----------|
| Positive control            | 5.48±0.5a  | 16.11±0.74a| 3.91±0.79a | 8.5  | ≥0.05    |
| *T. viride*                 | -190.16±0.82dA  | -251.19±2.17bAB | -289.07±1.11cB | -243.47 | <0.05    |
| *T. harzianum*              | -202.51±1.77d | -317.39±1.17b | -262.15±2.15c | -260.68 | <0.05    |
| *P. purpurascens*           | -18.38±0.39ab | -17.33±0.36a  | -18.8±0.51ab | -18.17 | ≥0.05    |
| *C. globosum*               | -30.79±0.47bc | -32.12±0.52a  | -33.01±0.54ab | -31.97 | ≥0.05    |
| *A. niger*                  | -32.34±0.69bc | -33.32±0.95a  | -36.19±0.56ab | -33.95 | ≥0.05    |
| *A. glaucus*                | -32.57±0.54bc | -33.39±0.45a  | -34.95±0.67ab | -33.64 | ≥0.05    |
| *Paecilomyces victoriae*    | -55.83±0.62cA | -45.99±0.28Aa  | -55.81±0.60Bb | -52.54 | <0.05    |
| Mean                        | -61.9      | -79.40     | -80.67     | nd   | nd       |
| P-value                     | <0.01      | <0.01      | <0.01      | nd   | nd       |

| Treatments                  | MT3        | MT4        | MT41       | Mean | P-value* |
|-----------------------------|------------|------------|------------|------|----------|
| Positive control            | 7.49±0.5a  | 11.11±0.74a| 3.15±0.75a | 7.25 | ≥0.05    |
| *T. viride*                 | -95.23±1.00e | -89.06±1.05d | -146.44±1.79b | -110.24 | ≥0.05    |
| *T. harzianum*              | -85.52±0.75e | -122.15±1.08e | -120.98±1.33b | -109.55 | ≥0.05    |
| *P. purpurascens*           | -34.77±0.5dc | -30.78±0.45c  | -34.35±0.54a  | -33.3  | ≥0.05    |
| *C. globosum*               | -16.36±0.47bc | -15.52±0.75bc | -24.89±1.14a  | -18.92 | ≥0.05    |
| *A. niger*                  | -22.95±0.63bc | -20.13±0.29bc | -17.32±0.33a  | -20.13 | ≥0.05    |
| *A. glaucus*                | -16.99±0.38bc | -16.63±0.28bc | -39.32±1.7a   | -24.31 | ≥0.05    |
| *Paecilomyces victoriae*    | -7.57±0.4bA | -6.69±0.35bA | -11.13±0.33aB | -8.46 | <0.05    |
| Mean                        | -30.21     | -32.21     | -43.48     | nd   | nd       |
| P-value                     | <0.01      | <0.01      | <0.01      | nd   | nd       |

* Duncan’s Multiple Range Test for percentage of *M. cannonballus* ascospores index mean in comparison among the three *M. cannonballus* isolates treated with three different antagonistic fungal spp.; small letters are for means comparison of the different antagonist’s in the same column.  
* Duncan’s Multiple Range Test is for percentage of *M. cannonballus* ascospores index mean in comparison among the seven antagonistic spp. for the different *M. cannonballus* isolates; capital letters are for comparison of means in the same row (Means of 6 plants per each replicates of three).  
* Probabilities associated with individual F tests. nd: not determined. After the initial (Pi = 5asc/g peat) and final (Pf) ascospores count, the following formula was applied to determine the percentage of the ascospore index (AI): AI (%) = (1-Pi/Pf) x 100

Biological control involves the use of one living organism to control another, and this management technology has received much attention in recent times. The number of biocontrol agents (BCAs) registered for use is relatively low, although their application was successful and proved to cause enhancement in crop growth (Ben Salem et al., 2016).

In the current study, in dual culture *in vitro* assays using several fungal genera such as; *Trichoderma*, *Penicillium*, *Chaetomium*, *Aspergillus* and *Paecilomyces* spp. as BCAs against the tested *M. cannonballus* isolates, revealed that *Trichoderma* spp. inhibited the growth of all these pathogenic isolates. In fact, the radial mycelial growth of the three *M. cannonballus* isolates decreased in presence of *T. viride* and *T. harzianum* with values ranging between 91.93 and 92.11%, respectively. These *in vitro* results on the efficacy of BCAs were similar to those reported by other authors such as Medeiros et
al., (2006). Zhang et al., (1999) reported that
*Trichoderma virens* exhibited *in vitro* antifungal activity by
inhibiting mycelial growth of *M. cannonballus* and
other soilborne pathogens such as *Didymella bryoniae, Macrophomina phaseolina and Phomopsis cucurbitae*. *T. album* isolates significantly
suppressed the growth of *M. cannonballus* and it
subsequently overgrew the pathogen (Zhang et al.,
1999), while, *Bacillus megaterium* was less
inhibitive (Mennatoullah et al., 2010).

El-Kolaly and Abdel-Sattar, (2013) revealed
that all the tested *Trichoderma* spp. inhibited the
growth of *Fusarium solani, M. cannonballus, Pythium aphanidermatum* and *Rhizoctonia solani*. *T. ressei* inhibited fungal growth significantly more
than the rest of isolates including; *T. pseudokoningii, T. viride* and *T. harzianum*. Recently, Rhouma et al.,
(2015) added that many microbial antagonists (i.e. *T. viride, T. harzianum, P. purpurascens, P. digitatum, A. flavus, A. niger, A. brevipes, A. glaucus, Gliocladium catenulatum* and *G. virens*) were prevalent in Tunisian cucurbit rhizosphere soil, and
possessed biocontrol activities against *M. cannonballus* with mycelial inhibition rate above
70%. Results of our study demonstrated that the
tested fungal bioagent’s modes of action in
confrontation with *M. cannonballus* include;
antibiosis, lysis of fungal cell wall, competition and
hyperparasitism in accordance with previous results
of Zhang et al., (1999).

Compared with the other BCA’s applied
preventively in the presence of *M. cannonballus; T. harzianum and T. virens* exerted highly significant
antagonistic potency, showing the lowest values of
disease incidence (8.33%), root disease index (0.80
and 0.79), ascospores dynamics population (1.54 and
1.5 asc/g of peat), and reduction of percent of *M.
cannonballus* ascospores (-260.68 and -243.47%),
respectively. Moreover, with their presence the
agronomic measurements have been enhanced with
respective values of plants height (76.75 and 79.83
cm), root volume (2.39 and 2.22 cm3), above ground
portions fresh wt. (16.57 and 16.07 g) and dry wt.
(2.49 and 2.6 g), underground portions fresh wt.
(0.654 and 0.725 g) and dry wt. (0.147 and 0.214 g),
respectively, compared with control treatments.

Current *in vivo* assay results were in accordance
with other studies such as Sanz et al., (1998), who
reported that *Trichoderma* spp. exhibited high
antifungal activity against *Monosporascus* sp. and
*Acremonium cucurbitacearum*. According to Zhang
et al., (1999), *T. virens* colonized the root systems of
muskmelon plants, significantly reduced *M.
cannonballus* colonization of roots, and suppressed
disease severity of seedlings by seed treatments. In
another study of El-Kolaly and Abdel-Sattar, (2013),
treatments with *T. harzianum* and *T. ressei* have not
only reduced the incidence of MRRVD, but also
reduced the *M. cannonballus* root invasion,
suggesting that BCAs were limiting the pathogen
infection. Ben Salem et al., (2016) pointed that
among six antagonists evaluated for biocontrol
potential against *M. cannonballus*, only *T. virens* and
*T. harzianum* significantly reduced disease incidence
and severity index after a preventive treatment
through soil drenching. Indeed, success of the
preventive applications could be attributed to the
hyperparasitism of the BCAs in the plant
rhizosphere, which inhibited the root infection by
soilborne pathogens and reduced their inoculums
build up (Rini and Sulochana, 2007). *Trichoderma*
spp. was demonstrated to have potential in *M.
cannonballus* disease management for *in vitro* and *in vivo* assays (Pastrana et al., 2016; Boughalleb-
M’Hamdi et al., 2018).

The increasing numbers of studies have
contributed to unveiling the molecular basis of the
plant-*Trichoderma* interaction, and the beneficial
effects of *Trichoderma* spp. to plants. Some selected
*Trichoderma* strains were shown to have direct
positive effects on plants such as; increasing their
growth potential and nutrient uptake, fertilizer use
efficiency, percentage and rate of seed germination,
in addition to stimulation of plant defences against
biotic and abiotic stresses. It has been reported by
Segarra et al., (2009) that *Trichoderma* spp. can
activate induced systemic resistance (ISR) in plants, a mechanism triggered after root colonization by nonpathogenic rhizobacteria or fungi and is regulated by a specific signal transduction cascade.

*Trichoderma* spp. are also known to produce a large number of antibiotics including; trichodermin, trichodermol, polyketides, peptaibols, sesquiterpenes, and steroids, all these active compounds are known to promote plant growth besides having biocontrol potential (Harman *et al*., 2004). Later, Müller *et al*., (2013) added that these fungi are prolific producers of a number of secondary metabolites with pharmaceutical and biotechnological significance that involve; non-ribosomal peptides, peptaibols, poliketides, pyrones, siderophores, beside volatile and non-volatile terpenes. For these reasons, they are major sources of many biofungicides and biofertilizers (Kaewchai *et al*., 2009). The germination of *M. cannonballus* ascospores and subsequent attachment to roots, occurs exclusively only in the cucurbits rhizosphere. According to Stanghellini *et al*., (2010), the interaction of *M. cannonballus* with susceptible cucurbits roots appears to be strongly related to the microbial composition in the rhizosphere.

**Conclusion**

*Trichoderma* spp. applied preventively and curatively showed significant effect on watermelon plants infested with *M. cannonballus*, and could be recommended for biocontrol use. *T. viride* and *T. harzianum* allowed not only the protection of plants, but also the improvement of the agronomic parameters including better axial growth and greater root biomass. Based on the current results, it is deduced that tested *T. viride* and *T. harzianum* could be employed in soil treatments as BCA’s to induce cucurbits systemic resistance, through a specific signal transduction cascade. The systemic resistance induction of cucurbits by *Trichoderma* spp. against *M. cannonballus* is a subject of future research.

**Conflict of interests**

The authors declare no conflict of interests regarding this article.

**Acknowledgements**

This research was supported by UR13AGR03, University of Sousse, Tunisia. The experiments comply with the current laws of the country in which they were performed.

**5. References**

Aegerter, B.J.; Gordon, T.R. and Davis, R.M. (2000). Occurrence and pathogenicity of fungi associated with melon root rot and vine decline in California. *Plant Disease*. 84: 224-230.

Aleandri, M.P.; Martignoni, D.; Reda, R.; Alfaro-Fernández, A.; Font, M.I.; Armengol, J. and Chilosi, G. (2017). Involvement of *Olpidium bornovanus* and *O. Virulentus* in the occurrence of melon root rot and vine decline caused by *Monosporascus cannonballus* in central Italy. *Journal of Plant Pathology*. 99: 169-176.

Ben Salem, I.; Rhouma, A.; Allagui, H. and Boughalleb M’hamdi, N. (2016). Mycoflora analysis and interaction study of pathogens antagonists fungi on watermelon development in Tunisia. *Faculty of Agriculture East Sarajevo Bosnia-Herzegovina*. 32: 1780-1789.

Ben Salem, I.; Armengol, J. and Boughalleb-Mhamdi, N. (2015a). Soil fungicide application in combination with grafting for the control of *Monosporascus* root rot and vine decline on cucurbits. *International Journal of Current Microbiology and Applied Sciences*. 9: 511-527.

Ben Salem, I.; M’Hamdi, M.; Armengol, J. and Boughalleb-Mhamdi, N. (2015b). Effects of crop sequences on soil population dynamics of *Monosporascus cannonballus* ascospores and *Monosporascus* Root Rot and Vine Decline.
incidence. International Journal of Current Microbiology and Applied Sciences. 9: 482-500.

Ben Salem, I.; Correia, K.C.; Boughabble, N.; Michereff, S.J.; León, M.; Abad-Campos, P.; García-Jiménez, J. and Armengol, J. (2013). *Monosporascus eutypoides*, a cause of *Monosporascus* root rot and vine decline in Tunisia, and evidence that *M. cannonballus* and *M. eutypoides* are distinct species. Plant Disease. 97: 737-743.

Boughalleb-M'Hamdi, N.; Ben Salem, I. and M'Hamdi, M. (2018). Evaluation of the Efficiency of *Trichoderma*, *Penicillium* and *Aspergillus* Species as Biocontrol Agents against Four Soil-borne Fungi of Melon and Watermelon. Egyptian Journal of Biological Pest Control. 28: 1-12.

Boughalleb-M'hamdi, N.; Rhouma, A.; Ben Salem, I. and M'hamdi, M. (2017). Screening of soil-borne fungal communities in relationship with organically amended soils and pathogenicity test of *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani*. Journal of Phytopathology and Pest Management. 1: 1-16.

Boughalleb, N.; Ben Salem, I.; Beltran, R.; Vicent, A.; Perez-Sierra, A.; Abad-Campos, P.; García-Jimenez, J. and Armengol, J. (2010). Occurrence of *Monosporascus cannonballus* in Watermelon Fields in Tunisia and Factors Associated with Ascospore Density in Soil. Journal of Phytopathology. 158: 137-142.

Cohen, R.; Pivonia, S.; Crosby, K.M. and Martyn, R.D. (2012). Advances in the biology and management of *Monosporascus* vine decline and wilt of melons and other cucurbits. Horticultural Reviews. 39: 77-120.

Cohen, R.; Burger, J.; Horev, C.; Koren, A. and Edelstein, M. (2007). Introducing grafted cucurbits to modern agriculture: The Israeli experience. Plant Disease. 91: 916-923.

Cohen, R.; Pivonia, S.; Edelstein, M.; Gamliel, A. and Katan, J. (2000). Toward integrated management of *Monosporascus* wilt of melon in Israel. Plant Disease. 84: 496-505.

El-Kolahy, G.A.A. and Abdel-Satta, M.A. (2013). Biological and Chemical Control of the Sudden Wilt Disease of Cantaloupe in Egypt. Journal of American Science. 9: 100-108.

Ferreira, A.C. (2011). Seleção de genótipos de cucurbitaceas a *Monosporascus cannonballus* e compatibilidade de porta-enxertos. Universidade Federal Rural do Semiárido Mossoró Brasil, pp. 61.

Fry, W.A. (1982). Principles of plant disease management. Academic Press, New York, USA, pp. 378.

Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I. and Lorito, M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. Nature Reviews Microbiology. 2: 43-56.

Heitholt, J.J. (1989). Water use efficiency and dry matter distribution in nitrogen and water stressed winter wheat. Agronomy Journal. 81: 464-469.

Hmouni, A.; Hajlaoui, M.R. and Mlaiki, M. (1996). Résistance de *Botrytis cinerea* aux benzimidazoles et aux dicarboximides dans les cultures abritées de tomate en Tunisie. EPPO Bulletin. 26: 697-705.

Kaechei, S.; Soytong, K. and Hyde, K.D. (2009). Mycofungicides and fungal biofertilizers. Fungal Diversity. 38: 25-50.

Medeiros, E.V.; Albuquerque, J.F.C.; Michereff, S.J.; Sales, J.R. and Nunes, G.H.S. (2006). Controle de *Monosporascus cannonballus* por tiazolidina-2,4-diona e efeito sobre o agente de controle biológico *Trichoderma* spp. Revista Caatinga. 19: 44-50.

Mennatoullah, Z.M.M.; Abdalla, M.Y.H.; El-Kassas, A.I.I. and Abd El-Hafez, A.A.E. (2010).
Comparative efficacy of chemical and biological methods against *Monosporascus cannonballus* in *vitro* and *in vivo*. Egyptian Journal of Biological Pest Control. 20: 125-129.

Mertely, J.C.; Martyn, R.D.; Miller, M.E. and Bruton, B.D. (1993). Quantification of *Monosporascus cannonballus* ascospores in three commercial muskmelon fields in south Texas. Plant Disease. 77: 766-771.

Müller, A.; Faubert, P.; Hagen, M.; Zu-Castell, W.; Polle, A.; Schnitzler, J.P. and Rosenkranz, M. (2013). Volatile profiles of fungi chemotyping of species and ecological functions. Fungal Genetics and Biology. 54: 25-33.

Musick, G.L.; Fairchild, M.L.; Fergusson, V.L. and Zuber, M.S. (1965). A method of measuring root volume in corn (*Zea mays* L.). Crop Science. 5: 601-602.

Pastrana, A.M.; Basallote-Ureba, M.J.; Aguado, A.; Akdi, K. and Capote, N. (2016). Biological control of strawberry soil-borne pathogens *Macrophomina phaseolina* and *Fusarium solani*, using *Trichoderma asperellum* and *Bacillus* spp. Phytopathologia Mediterranea. 55: 109-120.

Reda, R.; Aleandri, M.P.; Antonelli, M.; Varvaro, L.; Magro, P. and Chilosi, G. (2008). Monitoring and integrated management of melon collapse caused by *Monosporascus cannonballus*. Journal of Plant Pathology. 90: 419.

Rhouma, A.; Ben Salem, I.; M’hamdi, M. and Boughalleb-M’Hamdi, N. (2018). Relationship study among soils physico-chemical properties and *Monosporascus cannonballus* ascospores densities for cucurbit fields in Tunisia. European Journal of Plant Pathology. pp. 1-14.

Rhouma, A.; Ben Salem, I.; Allagui, H. and Boughalleb-M’hamdi, N. (2015). Etude de l’interaction entre les champignons antagonistes isolés à partir du sol et la mycoflore pathogène affectant la pastèque. 14ème symposium Internationales de Biotechnologie, Décembre 20-24, Association Tunisienne de Biotechnologie, Djerba, Tunisie. pp. 92.

Rini, C.R. and Sulochana, K.K. (2007). Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. Journal of Tropical Agriculture. 45: 21-28.

Rojo, F.G.; Reynoso, M.M.; Sofia, M.F. and Torres, A.M. (2007). Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. Crop Protection. 26: 549-555.

Sales, J.R.; Beltrán, R.; Vincent, A.; Armengol, J.; García-Jiménez, J. and Medeiros, E.V. (2007). Controle biológico de *Monosporascus cannonballus* com *Chaetomium*. Fitopatologia Brasileira. 32: 70-74.

Sanz, L.; Sales, R.J.; Armengol, J.; Monte, E.; García-Jimenez, J. and Grondona, I. (1998). Antagonismo de *Trichoderma* spp. frente a *Monosporascus* sp. *Acremonium cucurbitacearum* causantes do colapso en melon. In: IX Congresso de la Sociedad Espanola de Fitopatologia, Septiembre, Salamanca Espanha. pp. 287.

Segarra, G.; Van-der-Ent, S.; Trillas, I. and Pieterse, C.M.J. (2009). MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. Plant Biology. 11: 90-96.

Stanghellini, M.E.; Alcantara, P.T. and Ferrin, D.M. (2010). Germination of *Monosporascus cannonballus* ascospores in the rhizosphere: a host-specific response. Canadian Journal of Plant Pathology. 32: 402-405.
ascospores of *Monosporascus cannonballus*. Phytopathology. 90: 243-247.

Stanghellini, M.E. and Rasmussen, S.L. (1992). A quantitative method for the recovery of ascospores of *Monosporascus cannonballus* from field soil. Phytopathology. 82: 11-15.

Waugh, M.M.; Kim, D.H.; Ferrin, D.M. and Stanghellini, M.E. (2003). Reproductive potential of *Monosporascus cannonballus*. Plant Disease. 87: 45-50.

Zhang, J.X.; Bruton, B.D.; Howell, C.R. and Miller, M.E. (1999). Potential of *Trichoderma virens* for biocontrol of root rot and vine decline in *Cucumis melo* L. caused by *Monosporascus cannonballus*. Subtropical plant science. 51: 29-37.