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(RESEARCH ARTICLE)

Trichoderma harzianum: biocontrol to *Rhizoctonia solani* and biostimulation in *Pachyphytum oviferum* and *Crassula falcata*

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Abstract

In this study the possibility of using *Trichoderma harzianum* as a possible promoter of the growth of plants of *Pachyphytum oviferum* and *Crassula falcata* and in the biocontrol of *Rhizoctonia solani* was evaluated. The 2 experimental groups under cultivation were: 1) group without Trichoderma (CTRL), irrigated with water and previously fertilized substrate; 2) group with *Trichoderma harzianum* (TH) and fertilized substrate. The test showed a significant increase in the agronomic parameters analysed in plants treated with *Trichoderma harzianum* (TH). In fact, all plants treated with (TH) showed a significant increase in the number of leaves, plant height, vegetative and root weight, new shoots number, stem diameter, flowers and inflorescences number, flowering time. The use of *Trichoderma harzianum* can significantly influence photosynthesis and chlorophyll content in *Pachyphytum oviferum* and *Crassula falcata* and control the development of *Rhizoctonia solani*. The use of this antagonistic microorganism could therefore be a valid alternative for those growers who, during the cultivation cycle of ornamental and horticultural plants, pay attention to plant quality and respect for the environment, optimizing the use of fertilizers and reducing the application of plant protection products.

Keywords: Succulent; Biocontrol; Endophitism; Plant growth; Antagonism; Cactus

1. Introduction

Pachyphytum is a genus comprising a dozen short, sturdy species of shrubs with alternate leaves, succulent, often rosette-shaped. Bell-shaped flowers, red or white, similar to Echeveria, able to develop from only one side of the spike-shaped inflorescence. They originate in Mexico and are propagated by sucker, leaf and stem cuttings. The main species are *P. bracteosum, P. brevifolium, P. compactum, P. fittkaui, P. hookeri, P. longifolium, P. oviferum* [1].

Crassula comprises about 200 evergreen or deciduous species. Shrubs with succulent leaves, red star-shaped flowers, corimbiform. Native to South Africa, Namibia, Arabian Peninsula. The minimum cultivation temperature is 5°C. The rustic species resist well to temperatures of 2-3°C. Evergreen species have summer growth; deciduous species vegetate in winter. They propagate by seed and cutting [1].

The genus Trichoderma consists of anamorphic fungi isolated primarily from soil and decomposing organic matter, with teleomorphs, when known, belonging to the ascomycete genus Hypocrea (order Hypocreales). Fungal species belonging to this genus are worldwide in occurrence and easily isolated from soil, decaying wood and other plant organic matter. Trichoderma isolates are characterized by a rapid growth rate in culture and by the production of numerous spores (conidia) with varying shades of green. Their lifestyle is generally saprotrophic with minimal nutritional requirements; they are able to grow rapidly on many substrates, can produce metabolites with demonstrable antibiotic activity and may be mycoparasitic against a wide range of pathogens [2]. The abundance of

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Trichoderma spp. in various soils, coupled with a wide metabolic versatility, a dynamic colonization of plant rhizosphere and the ability to antagonize and repress a great number of plant pathogens are direct evidence of the role that these fungal species may play in biological control [3][4]. A number of isolates of Trichoderma have been found to be effective biocontrol agents of various soil-borne plant pathogenic fungi under greenhouse and field conditions. The knowledge of mechanisms of interaction of Trichoderma spp. with plant pathogenic fungi and the plant host is of importance to enhance the practical application of these beneficial microorganisms. They can work against fungal phytopathogens either directly through mechanisms such as mycoparasitism, competing for nutrients and space, modifying environmental conditions and antibiosis or indirectly promoting plant growth and plant defensive mechanisms. For all these reasons, the use of *Trichoderma spp.* strains as inoculants of substrates to be employed in nursery could confer an additional value both in order to control soilborne pathogens, to induce resistance or to promote growth of plants.

In this study the possibility of using *Trichoderma harzianum* as a possible promoter of the growth of plants of *Pachyphytum oviferum* and *Crassula falcata* and in the biocontrol of *Rhizoctonia solani* was evaluated.

2. Material and methods

2.1. Greenhouse experiment and growing conditions

The experiments, started at the beginning of February 2019, were carried out in CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on plants of *Pachyphytum oviferum* and *Crassula Falcata* (Figure). The plants were placed in pots ø 10 cm; 80 plants per thesis divided into 4 replicas of 20 plants each.

All plants were fertilized with the same amount of nutrients supplied through a controlled release fertilizer [4 kg m-3 Osmocote Pro® 6 months with 190 g/kg of N, 39 g/kg of P, 83 g/kg of K] mixed with the culture medium before transplanting.
The 2 experimental groups in cultivation were:

- group without Trichoderma (CTRL), irrigated with water and substrate previously fertilized;
- group with *Trichoderma harzianum* (TH) ($1x10^{11}$ cfu/Kg) and fertilised substrate. (dosage 200g per m^3 of substrate).

The plants were watered weekly for eight months.
On 5 September 2019, plant height, number of leaves, vegetative and root weight, number of new shoots, stem diameter, number of inflorescences and flowers, flower duration, Pn (LI-6400XT Portable Photosynthesis System, ten days before destructive analysis), chlorophyll content (FieldScout CM 1000 Chlorophyll Meter) were recorded. In addition, the number of plants out of the total affected by Rhizoctonia solani was evaluated.

Figure 1 Detail of *Crassula falcata* (A) and *Pachyphytum oviferum* (B) in greenhouse at CREA-OF in Pescia (PT)

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

3.1. Plant growth

The test showed a significant increase in the agronomic parameters analysed in plants treated with *Trichoderma harzianum* in both *Pachyphytum oviferum* and *Crassula falcata* plants. In fact, all plants treated with (TH) showed a significant increase in the leaves number, plants height, vegetative and radical weight, new shoots number, stem diameter, flowers and inflorescences number, flowering time.

In Pachyphytum, the number of leaves was 41.50 (TH) compared to 38.42 in the control (Figure 2A). The plant height was 48.46 cm (TH) compared to 41.96 cm for the untreated control (Figure 2B). There was also a significant increase in the vegetative weight, 190.62 g (TH) compared to 175.70 g of the control (Figure 2C), in the number of new shoots 2.50 (TH) compared to 1.08 (CTRL) (Figure 2D), in the stem diameter 2.56 (TH) compared to 1.92 (CTRL) (Figure 2E) and in the root weight 71.15 g (TH) compared to 66.68 g of the control (Figure 2F).

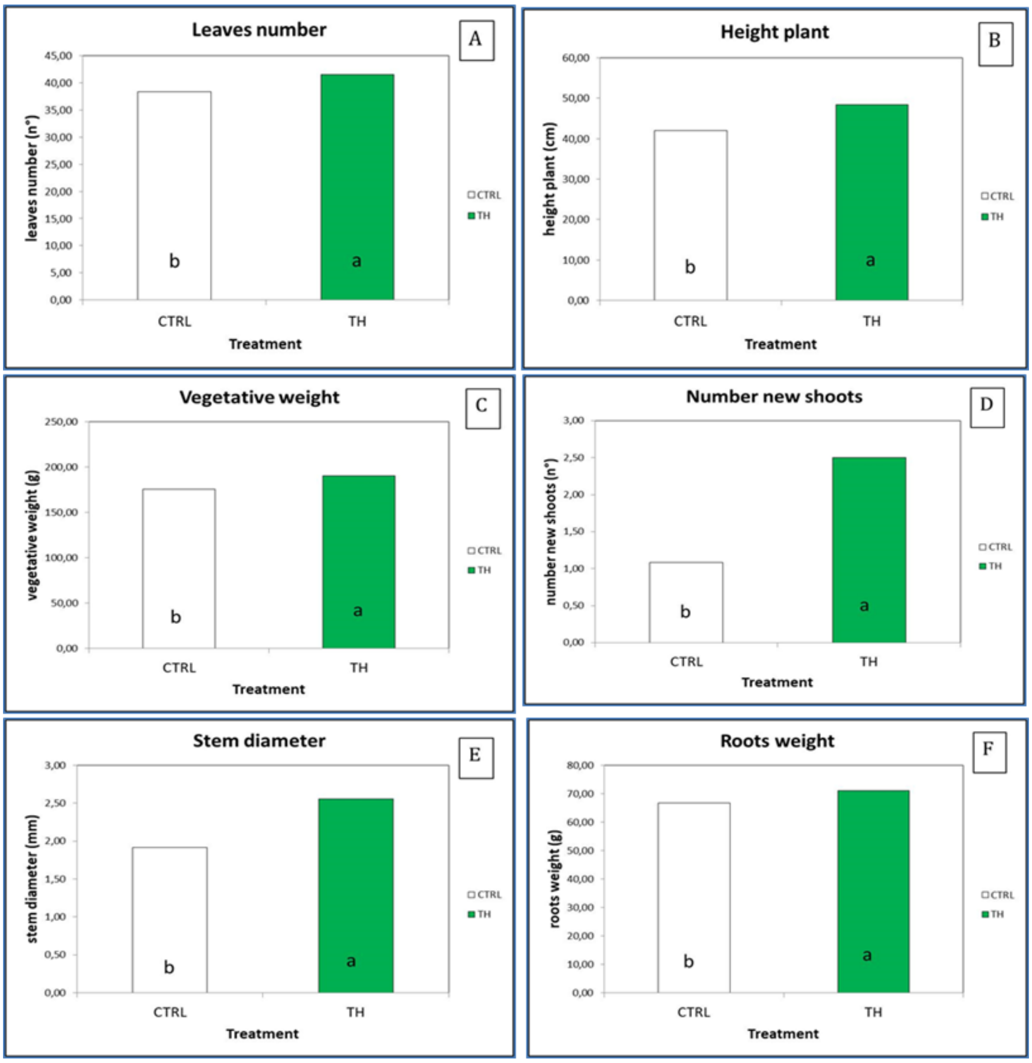

Figure 2 Effect of *Trichoderma harzianum* on growth improvement of *Pachyphytum oviferum.*

Legend: (A) leaves number; (B) height plant; (C) vegetative weight; (D) number new shoots; (E) stem diameter; (F) roots weight. Each value reported in the graph is the mean of four replicates ± standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test (P = 0.05).

The test also showed in *Crassula falcata* a significant increase in the number of leaves in (TH) 17.50 compared to 13.08 in the untreated control (Figure 3A). There was also a significant increase in plant height 17.28 cm (TH) compared to 12.57 in (CTRL) (Figure 3B), in the vegetative weight 208.72 g (TH) compared to 195.69 g (CTRL) (Figure 3C), in the number of inflorescences 2.58 (TH) compared to 1,50 of the untreated control (Figure 3D), in the number of flowers 39,17 (TH) compared to 33,00 of the untreated control (Figure 3E), in the roots weight 65,81 g (TH) compared to 58,84 of the untreated control (Figure 3F) and flower time 10,25 days (TH) compared to 7,50 (CTRL) (Figure 3G).

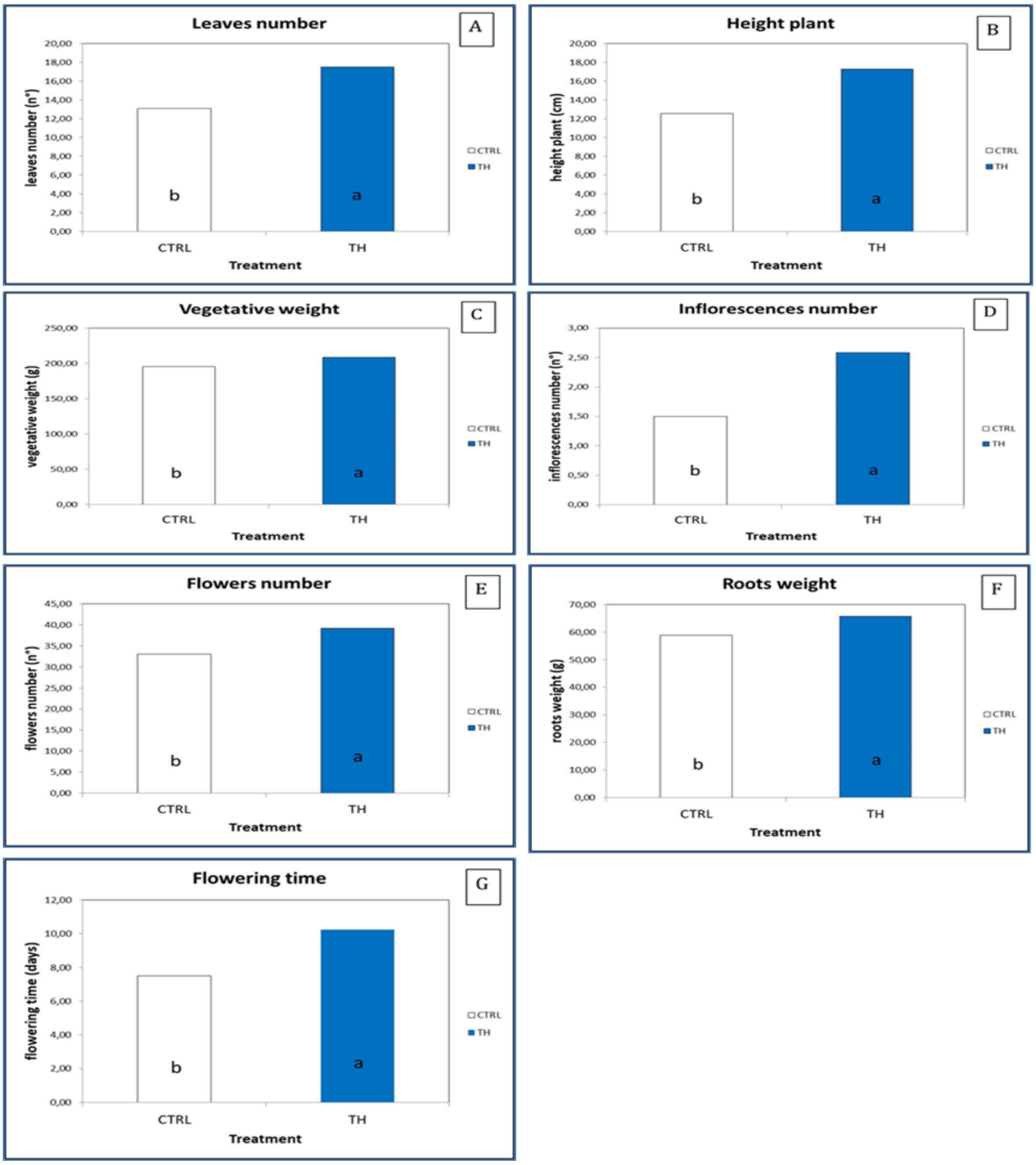

Figure 3 Effect of *Trichoderma harzianum* on growth and flowering improvement of *Crassula falcata.*

Legend: (A) leaves number; (B) height plant; (C) vegetative weight; (D) inflorescences number; (E) flowers number; (F) roots weight; (G) flowering time. Each value reported in the graph is the mean of four replicates ± standard deviation. Statistical analysis performed through one way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test (P = 0.05).

Figure 4 Improvement of vegetative growth in *Pachyphytum oviferum* after the treatment with *Trichoderma harzianum*

Figure 5 Improvement of vegetative growth in *Crassula falcata* after the treatment with *Trichoderma harzianum*

Table 2-3 show how the use of *Trichoderma harzianum* can significantly influence photosynthesis and chlorophyll content in *Pachyphytum oviferum* and *Crassula falcata*. In particular 12.66 µmol m-2 s-1 for (TH) compared to 11.39 (CTRL) (Table 1) and 12.33 µmol m-2 s-1 for (TH) compared to 11.15 µmol m-2 s-1 (CTRL) for plant photosynthesis (Table 2). Same trend for leaf chlorophyll content, 20.36 (TH) compared to 14.40 in (CTRL) (Table 1) and 17.89 (TH) compared to 13.18 in the untreated control (Table 2).

Table 1 Evaluation of the *Trichoderma harzianum* effect on the physiological parameters of *Pachyphytum oviferum*

Groups	**Pn (µmol m^{-2} s^{-1})**	**Chlorophyll content (spad index)**
CTRL	11,39 b	14,40 b
TH	12,66 a	20,36 a
ANOVA	***	***

Each value reported in the table is the mean of four replicates ± standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test (P = 0.05).

Table 2 Evaluation of the *Trichoderma harzianum* effect on the physiological parameters of *Crassula falcate*

Groups	**Pn (µmol m^{-2} s^{-1})**	**Chlorophyll content (spad index)**
CTRL	11,15 b	13,18 b
TH	12,33 a	17,89 a
ANOVA	***	***

Each value reported in the table is the mean of four replicates ± standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test (P = 0.05).

The evidence also shows that *Trichoderma harzianum* is antagonistic to *Rhizoctonia solani*, as the plants of *Pachyphytum oviferum and Crassula falcata* treated with the antagonist fungus were less affected by the pathogenic fungus.

Table 3 Evaluation of the effect of *Trichoderma harzianum* on biocontrol of *Rhizoctonia solani* on plants of *Pachyphytum oviferum* and *Crassula falcata* (percentage of plants affected).

Groups	*Pachyphytum oviferum* (%)	*Crassula falcata* (%)
CTRL	8,6	6,4
TH	2,2	1,7

4. Discussion

Treatments with *Trichoderma harzianum* have resulted in a significant improvement in the growth and flowering characteristics of *Pachyphytum oviferum* and *Crassula falcata*. In recent years, *Trichoderma spp.* have been widely used in agriculture as biocontrol agents and inoculants to provide plant growth promotion. They are involved in fundamental activities that ensure the stability and productivity of both agricultural and natural ecosystems. Some Trichoderma strains, described as rhizosphere competent and selectively used for commercial development, can cause an asymptomatic infection of roots, where the fungus colonization is limited to the outer cortical regions. These fungi behave as endophytes, colonizing the root epidermis and outer cortical layers and release bioactive molecules. At the same time, the transcriptome and proteome of plants are substantially altered. This intimate interaction with the plant provides a number of benefits only recently recognized for their variety and importance, including increased resistance of the plant to various biotic stresses through induced or acquired systemic resistance and to abiotic stresses such as water deficit/excess, high salinity and extreme temperature; enhanced nitrogen use efficiency by improved mechanisms of nitrogen reduction and assimilation and reduced overexpression of stress genes or accumulation of toxic compounds during plant response to pathogen. [5]

An additional benefit to consumer comes from an increased content of antioxidants in the fruit from plants treated by selected Trichoderma strains [6]. Moreover, it was also observed that the fertility of soils treated with some Trichoderma strains could be significantly improved beyond disease control, which increased the attractiveness of these fungi for a general use in crop production. As suggested also in this experiment with an increase in the content of chlorophyll and photosynthesis, the effect could be particularly strong in terms of root growth promotion, even though it has been not unusual to detect an increase in stem length and thickness, leaf area, chlorophyll content and yield (size and/or number of flowers or fruits) [7][8][9]. The molecular mechanisms supporting this highly desirable beneficial effect of plant growth promotion are not fully clarified and include improvement of nutrient availability and uptake for the plant [10][7]. Further analysis show a general increase in the absorption of many elements such as Pb, Mn, Zn, Al and the ability to solubilize some nutrients in the soil, such as phosphates, ions Fe3+, Cu2+, Mn4+, many times not easily available from the plant. [10]. Moreover, the involvement of growth phytormones from both plant and fungal origin could be involved in the phenomenon of plant growth promotion [11].

In combination with the direct effects on plant pathogens and with the ability of promote plant growth, *Trichoderma spp.* have also been found to stimulate plant defence mechanisms. In this test *Trichoderma harzianum* was able to control the development of *Rhizoctonia solani*. The presence of Trichoderma in plants involves an induction of resistance, often localized or systemic [7]. This phenomenon, also observed in field, has been attributed to a fungus-root biochemical cross talk involving many bioactive metabolites produced by the biocontrol agents [7][5][12]. Many Trichoderma strains colonize plant roots of dicots and monocots. During this process Trichoderma hyphae coil around the roots, form appressoria-like structures, and finally penetrate the root cortex. During the intercellular Trichoderma growth in the root epidermis and cortex the surrounding plant cells have been induced to deposit cell wall material and to produce phenolics compounds. This plant reaction limits the Trichoderma growth inside the root [11]. Effective Trichoderma strains are able to induce a stronger response in the plant compared to pathogen triggered immunity by producing a variety of microbe-associated molecular patterns (MAMP) as hydrophobins, expansin-like proteins, secondary metabolites, and enzymes having direct antimicrobial activity such as peroxidase, chitinase and glucanase. In addition, there is an accumulation of antimicrobial compounds and phytoalexins [6].

5. Conclusion

The test has shown that the use of *Trichoderma harzianum* can improve the growth and flowering of *Pachyphytum oviferum* and *Crassula falcata* plants, in particular by resulting in a significant increase in the vegetative and radical part of the plants, in the number of flowers, in the flower time, in the stems number, in the leaves number and in the plants height. The test also showed that *Trichoderma harzianum* is an excellent biocontrol agent, in this case against *Rhizoctonia solani*. This could be a valid alternative for those growers who, during the cultivation cycle of ornamental and horticultural plants, give attention to plant quality and respect for the environment. Trichoderma can optimize the use of fertilizers and reduce the application of plant protection products.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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