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(RESEARCH ARTICLE)

Gigaspora Margarita use to improve flower life in Notocactus and Gymnocalycium plants and roots protection against *Fusarium sp*.

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Abstract

The paper presents the results of research on cacti such as *Gymnocalycium baldianum, Gymnocalycium mihanovichii, Notocactus eugeniae* and *Notocactus leninghausii*, aimed at improving plant growth and defense against the pathogenic fungus *Fusarium sp.*, through the use of *Gigaspora margarita* inoculated in the growing medium.

Objectives of the work were: i) use *Gigaspora margarita* to assess if the use of arbuscular mycorrhizae can lead to an increase in the growth rate of Notocactus and Gymnocalycium, plants generally slow in their growth cycle; ii) to evaluate if the use of mycorrhizae can lead to an extension of the duration of the flower, to promote pollination of bees; iii) to assess if the use of *Gigaspora Margarita* allows greater protection of the roots against the fungus *Fusarium sp.* which often affects the roots of these cacti. The two experimental groups in cultivation were: i) group without arbuscular michorriza, irrigated with water and substrate previously fertilized; group with *Gigaspora Margarita*, irrigated with water and substrate previously fertilized.

All plants treated with *Gigaspora margarita* showed a significant increase in plant height and circumference, vegetative and root weight of the plants, number of new suckers, number of flowers and fruits and flower life. In addition, there was significant control of the fungus *Fusarium sp.* in plants in which *Gigaspora margarita* was inoculated into the substrate, in fact a reduced infection of the roots due to this pathogen was found. The application of mycorrhiza in the cultivation of plants can guarantee the possibility to obtain a higher quality product, a higher resistance to biotic stress, an increase in growth rate, very interesting aspects in the succulent and cactus plants sector.

Keywords: Sustainable applications; Biostimulant; Microorganisms; Cactus; Arbuscular Mycorrhiza

1. Introduction

An arbuscular mycorrhiza is characterized by a type of mycorrhiza in which the symbiont fungus (AM fungus, or AMF) penetrates the cortical cells of the roots of a vascular plant that forms arbuscules [1]. Arbuscular mycorrhizae involve the formation of special structures, arbuscules and vesicles by phylum Glomeromycota fungi. AM fungi improve the ability of plants to capture nutrients such as phosphorus, sulfur, nitrogen and micronutrients from the soil [2]. The evolution of the arbuscular mycorrhizal symbiosis seems to have played a fundamental role in the initial colonization of the soil by plants and in the evolution of vascular plants. This kind of symbiosis is a highly evolved mutualistic relationship found between fungi and plants, the most prevalent known plant symbiosis and AMF is found in 80% of today's vascular plant families [3,4]. The enormous advances in research on mycorrhizal physiology and ecology over the last 40 years have led to a greater understanding of the multiple roles of MFA in the ecosystem. This knowledge can benefit man in the management of the ecosystem, in restoring the balance of soil characteristics and obvious to improve

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the quality of plants grown in agriculture [5]. In parenchyma, the fungus forms highly branched structures for the exchange of nutrients with the plant called arbuscules. These are the distinctive structures of the mycorrhizal arbuscular fungus. The arbuscules are the exchange sites for phosphorus, carbon, water and other nutrients [6]. The host plant exerts a control over the intercellular hyphal proliferation and arbuscule formation. There is a decondensation of the plant's chromatin, which indicates increased transcription of the plant's DNA in arbuscule-containing cells [7]. Major modifications are required in the plant host cell to accommodate the arbuscules. The vacuoles shrink and other cellular organelles proliferate. The plant cell cytoskeleton is reorganized around the arbuscules [8].

Several arbuscular mycorrhizal fungi have been found to control soil pathogens such as *Aphanomyces, Cylindrocladium, Fusarium, Macrophomina, Phytophthora, Pythium, Rhizoctonia, Sclerotinium* and *Verticillium* species. For example in greenhouse conditions *Glomus fasciculatum* and *Gigaspora margarita* have been shown to decrease radical rot diseases caused by *Fusarium oxysporum* f.sp. *asparagus* in asparagus and *Glomus clarum* has been shown to decrease radical necrosis due to *Rhizoctonia solani* in cowpea [9,10,11] .

Notocactus and Gymnocalycium are genera of the exclusively South American tribus Notocacteae. The taxonomy of the genus Notocactus is controversial. While Backeberg (1977) divided the genus into Brasilicactus, Eriocactus, Notocactus and Wigginsia, Taylor (1989) and Hunt & Taylor (1990) proposed a merger of the genus Notocactus with Parodia. The genus Notocactus includes subtropical and temperate South American lowland species east of the Andes. The distribution area of the genus Gymnocalycium is similar to that of Notocactus but Gymnocalycium species are also found in Paraguay and Bolivia. Each plant of Notocactus and Gymnocalicium produces only a few buds that flower only for a short period of time. Therefore it could be assumed that mainly polyvalent bees temporarily feed the cactus flowers. In order to specialize or to become oligoleptic on cactus flowers, bees must combine the problem of timing their foraging activity with short flowering periods. Currently there is little information about flower visitors and pollinators of the Notocactus and Gymnocalycium genus cactus pollinators [12,13].

In this experiment, the main objective was to:

- Use *Gigaspora margarita* to assess whether the use of shrubby mycorrhizae can lead to an increase in the growth rate of Notocactus and Gymnocalycium, plants generally slow in their growth cycle;
- Evaluate whether the use of mycorrhizae can lead to an extension of the flower duration, to promote pollination of bees;
- Evaluate if the use of *Gigaspora Margarita* allows a greater root protection from *Fusarium sp.* that frequently affects the roots of these cacti.

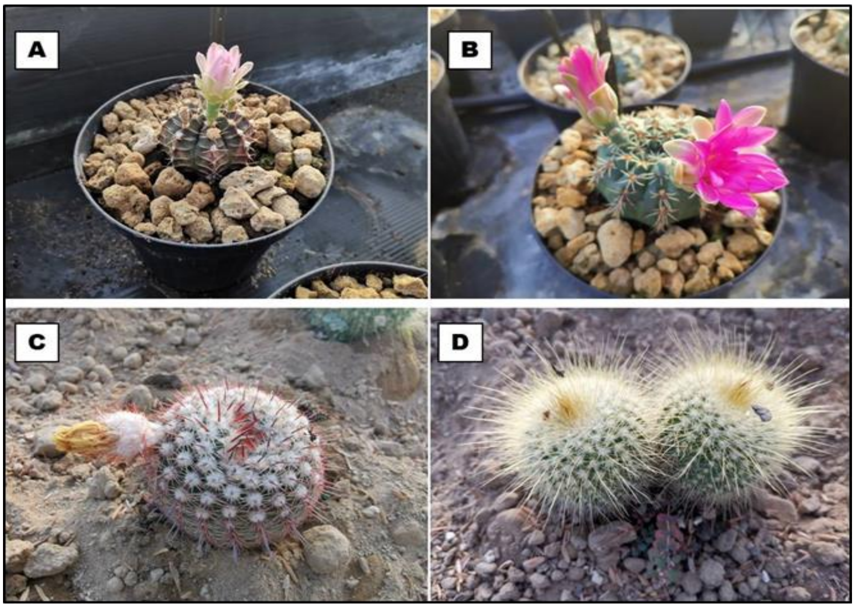

Figure 1 Detail of *Gymnocalycium mihanovichii* (A), *Gymnocalycium baldianum* (B), *Notocactus eugeniae* (C) and *Notocactus leninghausii* (D) in greenhouse

2. Material and methods

2.1. Greenhouse experiment and growing conditions

The experiments, started in January 2020, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Gymnocalycium baldianum, Gymnocalycium mihanovichii*, *Notocactus eugeniae* and *Notocactus leninghausii*. (Figure 1A,1B,1C,1D)

The plants were placed in ø 12 cm pots; 45 plants per thesis, divided into 3 replicas of 15 plants each. All plants were fertilized with a controlled release fertilizer (2 kg m^{-3} Osmocote Pro®, 6 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting.

The two experimental groups in cultivation were:

- Group without arbuscular mychorriza (CTRL) (peat 50% + pumice 30%+sand 10%+zeolite 10%), irrigated with water and substrate previously fertilized;
- Group with *Gigaspora margarita* (GM) (peat 50% + pumice 30% + sand 10%+zeolite 10%), irrigated with water and substrate previously fertilized. *Gigaspora maragarita* has been isolated by TNC Mycorr MAX (1.2 x10^4 spores/Kg)

The plants were watered 1 time a week and grown for 10 months. The plants were irrigated with drip irrigation. The irrigation was activated by a timer whose program was adjusted weekly according to climatic conditions and the fraction of leaching. On October 2, 2020, plant circumference, plant height, the vegetative and root weight, number of new sucker, flowers and fruits number, flower duration. In addition, the number of plants that have been infected at root level by *Fusarium sp.* has been evaluated.

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 agronomic parameters analyzed in plants treated with Gigaspora margarita on *Gymnocalycium baldianum, Gymnocalycium mihanovichii, Notocactus eugeniae* and *Notocactus leninghausii*.

In fact, all plants treated with Gigaspora margarita (GM) showed a significant increase in plant height and circumference, vegetative and root weight of the plants, number of new suckers, number of flowers and fruits and flower life. In addition, there was a significant control of the fungus Fusarium sp. in plants where Gigaspora margarita was inoculated in the substrate, in fact a reduced mortality due to this pathogen was found.

In particular in *Gymnocalycium baldianum* (Table 1), the treatment with G. margarita (GM) significantly improved the height of the plants, 6.40 cm (GM) compared to 5.28 cm (CTRL). It significantly increased the circumference of the plants 11.42 cm (GM) compared to 10.72 cm (CTRL) of the untreated control. There was also an increase in vegetative weight, 39.14 g (GM) compared to 35.60 g (CTRL) and root weight 26.60 g (GM) compared to 23.06 g of the control.

The test also showed a significant increase in sucker number 1.20 (GM) compared to 0.40 (CTRL), flower number 3.80 (GM) compared to 2.63 (CTRL), fruit number 1.65 (GM) compared to 0.41 (CTRL) and flower life 3.41 (GM) compared to 2.43 days of untreated control.

Similarly in *Gymnocalycium mihanovichii* (Table 2), the treatment with Gigaspora margarita (GM) showed a significant increase in plant height of 6.22 cm, compared to 5.06 cm of the control (CTRL). The treatment with arbuscular mycorrhiza also improved the circumference of the plants, 10.08 cm (GM) compared to 9.38 cm (CTRL). There was also a significant increase in vegetative weight, 40.24 g (GM) compared to 38.46 g (CTRL) and root weight 32.16 g (GM) compared to 27.38 g of untreated control (Figure 3). It also significantly increased the number of flowers 4.80 (GM) compared to 3.82 (CTRL), the number of fruits, 1.63 (GM) compared to 0.61 of the control and flower life 3.21 days in (GM), compared to 2.24 days of the untreated control. There are no significant differences in the number of new suckers between the two treatments.

In Notocactus eugeniae (Table 3), plants treated with Gigaspora margarita showed a significant increase in plant height 6.12 cm (GM), compared to 5.24 cm (CTRL). In addition there is a significant increase in the circumference of the plants, 9.21 cm (GM) compared to 8.64 cm (CTRL). Also on N. eugeniae there is an increase in vegetative weight 38.76 g (GM) compared to 36.31 g (CTRL) and root weight 25.64 g (GM) compared to 23.52 g (CTRL) (Figure 2). It was also evident the significant increase in the number of suckers in plants treated with Gigaspora margarita, 1.21 (GM) compared to 0.27 (CTRL), the increase in the number of flowers 2.26 in (GM) compared to 1.28 of the untreated control, the number of fruits 1.46 in (GM) compared to 0.41 (CTRL) and the flower life 3.64 days in (GM) compared to 2.42 days of the control.

In *Notocactus leninghausii* (Table 4), plants treated with Gigaspora margarita have shown a significant improvement in plant height 6.42 cm (GM), compared to 6.05 cm (CTRL). Moreover there was a significant increase in the circumference of the plants, 8.27 cm (GM) compared to 7.34 cm (CTRL). Even on N. leninghausii there was an increase in vegetative weight 33.92 g (GM) compared to 32.04 g (CTRL) and root weight 23.78 g (GM) compared to 21.74 g (CTRL) (Figure 2,4). It was also relevant the increase in the number of suckers in plants treated with Gigaspora margarita, 1.46 (GM) compared to 0.61 (CTRL), the increase in the number of flowers 2.03 in (GM) compared to 1.11 of the untreated control, the number of fruits 2.44 in (GM) compared to 0.58 (CTRL) and the flower life 3.46 days in (GM) compared to 2.28 days of the control.

There was also a reduction in mortality due to Fusarium sp. in plants treated with Gigaspora margarita (Table 5).

In *Gymnocalycium baldianum*, 0.78 plants (GM) compared to 3.61 in (CTRL), in *Gymnocalycium mihanovichii* 0.21 plants (GM) when compared to 2.84 in the control, in *Notocactus eugeniae* 0.72 plants (GM) instead of 2.46 in the control, in *Notocactus leninghausii* 0.21 plants (GM) compared to 0.84 plants in the untreated control.

Table 1 Evaluation of *Gigaspora Margarita* on agronomic characters on plants of *Gymnocalycium baldianum*.

Groups	Plant height (cm)	Plant circumference (cm)	Vegetative weight (g)	Roots weight (g)	Suckers number (n°)	Flowers number (n°)	Fruits number (n°)	Flower life (days)
CTRL	5,28 b	10,72 b	35,60 b	23,06 b	0,40 b	2,63 b	0,41 b	2,43 b
GM	6,40 a	11,42 a	39,14 a	26,60 a	1,20 a	3,80 a	1,65 a	3,41 a
ANOVA	***	*	***	***	*	**	**	*

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).
Legend: (CTRL) control; (GM) *Gigaspora margarita.*

Table 2 Evaluation of *Gigaspora margarita* on agronomic characters on plants of *Gymnocalycium mihanovichii.*

Groups	Plant height (cm)	Plant circumference (cm)	Vegetative weight (g)	Roots weight (g)	Suckers number (n°)	Flowers number (n°)	Fruits number (n°)	Flower life (days)
CTRL	5,06 b	9,38 b	38,46 b	27,38 b	0,24 a	3,82 b	0,61 b	2,24 b
GM	6,22 a	10,08 a	40,24 a	32,16 a	1,00 a	4,80 a	1,63 a	3,21 a
ANOVA	**	***	**	***	**ns**	**	*	**

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).
Legend: (CTRL) control; (GM) *Gigaspora margarita.*

Table 3 Evaluation of *Gigaspora margarita* on agronomic characters on plants of *Notocactus eugeniae.*

Groups	Plant height (cm)	Plant circumference (cm)	Vegetative weight (g)	Roots weight (g)	Suckers number (n°)	Flowers number (n°)	Fruits number (n°)	Flower life (days)
CTRL	5,24 b	8,64 b	36,31 b	23,52 b	0,27 b	1,28 b	0,41 b	2,42 b
GM	6,12 a	9,21 a	38,76 a	25,64 a	1,21 a	2,26 a	1,46 a	3,64 a
ANOVA	***	**	**	***	**	**	*	**

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).
Legend: (CTRL) control; (GM) *Gigaspora margarita.*

Table 4 Evaluation of *Gigaspora margarita* on agronomic characters on plants of *Notocactus leninghausii*

Groups	Plant height (cm)	Plant circumference (cm)	Vegetative weight (g)	Roots weight (g)	Suckers number (n°)	Flowers number (n°)	Fruits number (n°)	Flower life (days)
CTRL	6,05 b	7,34 b	32,04 b	21,74 b	0,61 b	1,11 b	0,58 b	2,28 b
GM	6,42 a	8,27 a	33,92 a	23,78 a	1,46 a	2,03 a	2,44 a	3,46 a
ANOVA	**	***	**	***	*	*	**	**

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).
Legend: (CTRL) control; (GM) *Gigaspora margarita.*

Table 5 Plants number affected by *Fusarium sp.*

Plants	*Gymnocalycium baldianum*	*Gymnocalycium mihanovichii*	*Notocactus eugeniae*	*Notocactus leninghausii*
Treatments	X	X	X	X
CTRL	3,61 a	2,84 a	2,46 a	0,84 a
GM	0,78 b	0,21 b	0,72 b	0,21 b
ANOVA	***	***	***	*

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).
Legend: (CTRL) control; (GM) *Gigaspora margarita*; (X) plants infected by *Fusarium sp.*

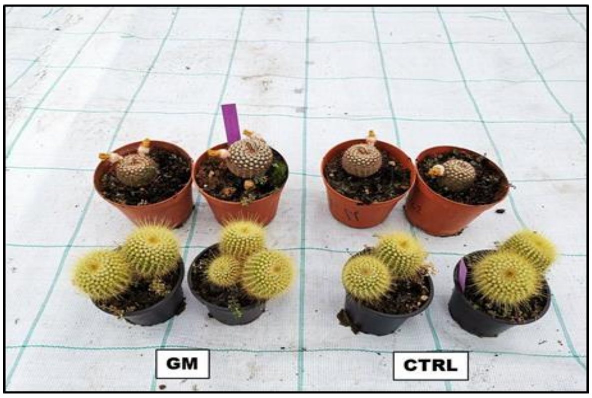

Figure 2 Effect of *Gigaspora margarita* on vegetative biomass of *Notocactus eugeniae* and *Notocactus leninghausii*.
Legend: (CTRL) control; (GM) *Gigaspora margarita*

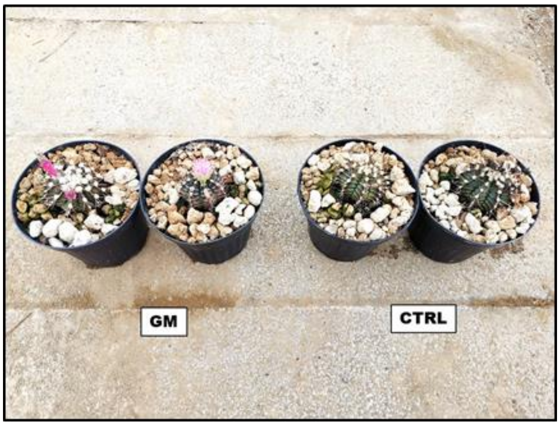

Figure 3 Effect of *Gigaspora margarita* on vegetative biomass of *Gymnocalycium mihanovichii*.
Legend: (CTRL) control; (GM) *Gigaspora margarita*

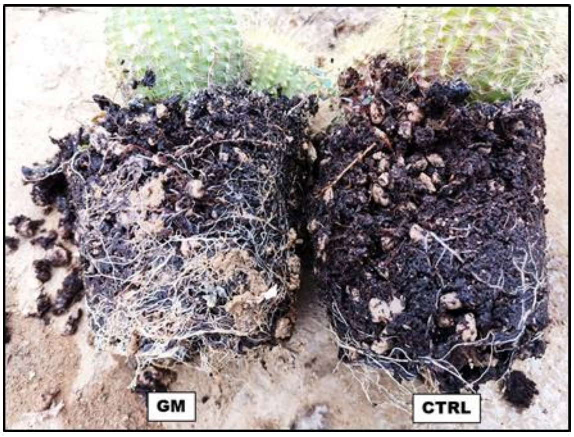

Figure 4 Effect of *Gigaspora margarita* on roots biomass of *Notocactus leninghausii*.
Legend: (CTRL) control; (GM) *Gigaspora margarita*

4. Discussion

The association between the fungal hyphae and the radical organs of plants represents a symbiosis that in 1885 Frank called mycorrhiza, meaning by this term a kind of new organ with its own shape and a certain physiology [14].

A wide range of relationships can be established between plant roots and fungi. In these relationships the plant does not show pathological symptoms due to the presence of fungal organisms. The classification of mycorrhizae is based both on morphological aspects and on where the fungus is located. Mycorrhiza is mainly established on the lateral roots and branches. Mycorrhizate roots remain shorter and tend to have a larger diameter. The external appearance varies depending on the type of fungus, the intensity of the infection and the way the root system of the plant grows [15].

The intensity of mycorrhizal infection varies from soil to soil. The amount of roots is higher in acid humus mor soils than in mull soils. The formation of mycorrhizal roots is favoured by conditions of nutrient deficiency, especially nitrogen, as well as intense photosynthetic activity. It seems therefore that the carbohydrate content of the roots is a factor of decisive importance and that any condition that favors the presence of an excess of carbohydrates stimulates mycorrhizal infection [16].

Mycorrhizal roots have a higher capacity to absorb mineral elements, especially nitrogen and phosphorus, than normal roots. This capacity, useful in poor soils, is favored by a greater absorbing surface area, also because from the fungal sheath branch off mycelial filaments that penetrate the surrounding soil. In addition, it seems that the fungus carries out a very intense metabolic activity and that this activity contributes to the mobilization of nutrients.

As far as nitrogen is concerned, there are good reasons to believe that the fungus absorbs organic nitrogen compounds from the soil and, after metabolizing them, releases the mineralized nitrogen to the plant [17].

In this test, plants treated with *Gigaspora margarita* showed a significant increase in plant height and circumference, vegetative and root weight, the number of suckers of flowers and fruits and also significantly increased the longevity of the flowers.

Very interesting aspect is also the fact that the use of arbuscular mycorrhizae on cacti, in particular Gymnocalycium and Notocactus can control the development of *Fusarium sp.*

Mycorrhizae are of undoubted interest, since they have a sometimes decisive significance in some silvicultural problems, such as the introduction of new plant species, reforestation, renewal and nursery production. However, they can also play an important role on cacti [16]. The inoculation of the soil and substrates can be done with fungus cultures produced in the laboratory, or with soil from forests where the presence of specific mycorrhizae is known.

5. Conclusion

The test showed how the use of *Gigaspora margarita* in growing media can improve the quality and growth of Gymnocalycium and Notocactus cactus plants. In particular through increasing plant height and circumference, vegetative and root weight, the suckers, flowers and fruits number and moreover significantly increased the life of the flowers. The use of this mycorrhiza, in addition to accelerating the growth cycle of cacti, has also increased the life time of the flowers, which is very important to increase the chances of pollination by insects. Another very interesting aspect concerns the biocontrol by *Gigaspora margarita* against *Fusarium sp*., the plants in which the mycorrhiza has been inoculated in the substrate have shown a reduced presence of the fungus pathogen in the roots.

The application of mycorrhiza in the cultivation of plants can guarantee the possibility to obtain a higher quality product, a higher resistance to biotic stress, an increase in growth rate, very interesting aspects in the succulent and cactus plants sector.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declares no conflict of interest.

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