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Myrtillocactus geometrizans fruit plant stimulated with Effective microorganisms

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Abstract

The aim of this work is to develop an innovative technology for the cultivation of *Myrtillocactus geometrizans*, introducing the use of Effective microorganisms and at the same time, limiting the use of mineral fertilizers, plant protection products and improving the physico-chemical and organoleptic characteristics of garambullos for consumption and processing. The trial showed a significant improvement in the agronomic parameters analysed on *Myrtillocactus geometrizans* plants treated with Effective microorganisms. In particular, there was an increase in plant height and circumference, vegetative and root weight, number of flowers and fruits, number and length of thornes in plants treated with microorganisms. In addition, the use of EM microorganisms showed a significant increase in total betalains, ascorbic acid, phenols and total flavonoids in garambullos. It was important to underline how the use of Effective microorganisms guaranteed, despite the reduction of irrigation and fertilisation by 50% in the growing medium, the same results in terms of agronomic parameters and fruit production and quality as the control with irrigation and fertilisation under optimal conditions.

The application of Effective microorganisms in agricultural processing can therefore guarantee higher production standards, with a possible reduction in costs fertilizer and water. Particularly for those farms that want to focus on the production of ornamental and fruit cacti. Fruits obtained from growing plants treated with Effective microorganisms have a high antioxidant and nutraceutical potential, which is very important especially in this age where food is also a medicine.

Keywords: Cactus; Effective microorganisms; Sustainable agriculture; Fruit plants; Garambullos

1. Introduction

The botanist Michelangelo Console, from Palermo, created the name of the genus by referring to the similarity of the fruits with those of the blueberry. These plants are native to the central highlands of Mexico, from the state of San Luis Potosì to Oaxaca. In its natural environment this plant is almost tree-like, reaching 4 m in height with a well-defined and branching trunk with upward curved articles; but, as happens with many species, the plants in cultivation, obtained from cuttings, do not develop a trunk and branch out from the base, issuing new stems of 6-10 cm in diameter, green-blue and strongly glaucous due to the pruine covering them in the young part. The ribs are 5-6, wide at the base, with widely spaced areoles and only slightly felted. The radial spines are 5, and even more in the old specimens, about 2 mm long, reddish-brown initially; there is only one blackish central spine, 2-7 cm long, flat laterally. The flowers are 2.5-3.5 cm wide, with a very short tube and greenish perianth segments; several flowers may appear simultaneously from each areola. The fruits are globular, 1-2 cm large, bluish [1, 2].

The fruits (garambullos) are consumed fresh or processed in different forms such as jams or jellies and is highly appreciated for its organoleptic characteristics [3, 4, 5]. However, there is still limited literature exists on the

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physicochemical and antioxidant properties of the fruit. However, from the information that does exist on garambullo fruits, it is mentioned that they are rich in photochemicals such as phenolic compounds, vitamin C and betalains, which give the fruit good functional properties [6].

Effective micro-organisms are a commercial microbial selection containing a mixture of coexisting beneficial micro-organisms collected from the natural environment. This selection was developed at the University of Ryukyus, Japan, in early 1980 by Prof. Teruo Higa. About 80 different microorganisms are able to positively influence the decomposing organic substance in such a way as to transform it into a process of "promoting life".

The main species involved in EM include [7, 8]:

- Lactic acid bacteria – *Lactobacillus plantarum, L. casei, Streptoccus lactis*;
- Photosynthetic bacteria – *Rhodopseudomonas palustrus, Rhodobacter spaeroides* [9];
- Yeast – *Saccharomyces cereviasiae, Candida utilis*;
- Actinomycetes – *Streptomyces albus, S. griseus*;
- Fermenting fungi – *Aspergillus oryzae, Mucor hiemalis*.

EM is a fermented mixed culture of naturally occurring species of micro-organisms coexisting in an acid environment (pH less than 3, 5). Micro-organisms in EM improve crop health and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, accelerating the decomposition of organic materials and controlling soil diseases. Effective micro-organisms can be used as herbal insecticides to control insects and pathogenic microorganisms and can also be used as plant growth inducers. Soil micro-organisms have an important influence on soil fertility and plant health [10, 11]. EMs interact with the soil-plant ecosystem by controlling plant pathogens and disease agents, solubilising minerals, increasing plant energy availability, stimulating the photosynthetic system, maintaining the microbiological balance of the soil, fixing biological nitrogen [12]. A characteristic of this mixture is the coexistence of aerobic and anaerobic micro-organisms. After Higa's research in Japan [7], the characteristics of EM have been studied in many countries. Studies have shown positive effects of the application of EM on soils and plants on soil quality and nutrient supply [13], plant growth [14,15], crop yield [16] and crop quality [17,18]. However, in some studies no positive effects were found [19, 20].

The aim of this work is to develop an innovative technology for the cultivation of *Myrtillocactus geometrizans*, introducing the use of Effective microorganisms and at the same time, limiting the use of mineral fertilizers and plant protection products. Furthermore, the aim of the study was to determine the effect of applying EM in improving the cultivation of *Myrtillocactus geometrizans* on the physico-chemical and organoleptic characteristics of garambullos for consumption and processing.

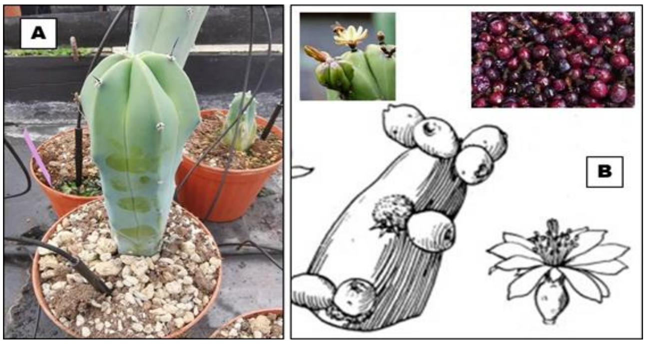

Figure 1 Particulars of *Myrtillocactus geometrizans* plant (A), flowers and fruits (garambullos) (B)

2. Material and methods

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 *Myrtillocactus geometrizans* (). The plants were placed in ø 12 cm pots; 30 plants per thesis, divided into 3 replicas of 10 plants each. All plants were fertilized with a controlled release fertilizer (2 kg m^{-3} Osmocote Pro®, 9-12 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting. The experimental groups were:

- Group without Effective microorganisms (CTRL100) (peat 50% + pumice 50%), irrigated with water (100%) and substrate previously fertilized (100%);
- Group without Effective microorganisms (CTRL50) (peat 50% + pumice 50%) irrigated with water (50%) and substrate previously fertilized (50%);
- Group with Effective microorganisms (EM100) (peat 50% + pumice 50%) irrigated with water (100%) and substrate previously fertilized (100%), dilution 1:100 (1L of EM inoculum dilution 1:100 was used for each 10L of peat);
- Group with Effective microorganisms (EM50) (peat 50% + pumice 50%) irrigated with water (50%) and substrate previously fertilized (50%), dilution 1:100 (1L of EM inoculum dilution 1:100 was used for each 10L of peat);

The plants were watered 2 times a week in the CTRL100 and EM100 and grown for 12 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 January 11, 2021, plant height, plant circumference, flowers number, thorns number, thorns lenght, garambullos (fruit) production per plant, vegetative weight, roots weight. In addition total phenols, flavonoids and betalains, ascorbic acid of garambullos was analysed.

2.1. Ascorbic acid content

Ascorbic acid was determined using the modified method of Dürüst et al. [21]. A 0.1 g of sample was weighed and 10 mL of 3 % (v/v) metaphosphoric acid solution was added. The samples were vortexed for 30 s and centrifuged at 10000 ×g for 10 min at 5 °C. From the supernatant 2 mL were taken from the supernatant and 2 mL of the buffer system for pH = 4 (glacial acetic acid: sodium acetate 5 % (w/v), 1:1), 3 mL dichloroindophenol and 15 mL xylene, then shaken vigorously. The absorbance was read in the spectrophotometer (Spectrophotometer model Onda UV-21) at 520 nm. Results were expressed as mg ascorbic acid per kg fresh weight (FP) [4].

2.2. Betalaines content

The concentration of betalains was determined spectrophotometrically with 0.1 g of freeze-dried fruits were taken and mixed with 10 mL of 20% (v/v) methanol. The samples were vortexed for 30 s and centrifuged at 10000 ×g for 10 min at 5 °C. The absorbance of the supernatants was measured at 537, 476 and 600 nm (Spectrophotometer model Onda UV-21) [4].

The content of total betalains (TBC) was calculated by the equation: TBC [mg / L] = [(A × DF × MW × 1000 / å × l), where A is the absorbance value, DF × MW × 1000 / å × l), where A is the absorbance value, DF × MW × 1000 / å × l). value of the absorbance, DF is the dilution factor and l is the cell length (1 cm). For the quantification of betacyanins and betaxanthins, the molecular weights (MW) and the molar extinction coefficients (ε) of betanin (MW = 550 g / mol; ε = 60 000 L / mol cm in H2O; absorption maxima =537 nm) and indicaxanthin (MW = 308 g / mol; ε = 48 000 L / mol cm in H2O; absorption maximum = 476 nm) [4].

2.3. Total phenols and flavonoids

Total phenols were determined spectrophotometrically following the method of Singleton and Rossi [22] taking 0.5 mL of the methanol supernatant and adding 0.5 mL of Folin diluted 50% with distilled water, 1.5 mL of a 2% sodium carbonate solution and 2.5 mL of distilled H2O were added. The results were expressed as mg gallic acid equivalents (EAG)/Kg fresh weight. Flavonoids were determined following the method described by Rosales et al. (2011). The results are reported as mg quercetin equivalents (EQ)/Kg fresh weight [4].

2.4. 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

The trial showed a significant improvement in the agronomic parameters analysed on *Myrtillocactus geometrizans* plants treated with Effective microorganisms (EM). In particular, there was an increase in plant height and circumference, vegetative and root weight, number of flowers and fruits, number and length of thornes in plants treated with microorganisms. In addition, the use of EM microorganisms showed a significant increase in total betalains, ascorbic acid, phenols and total flavonoids in garambullos. It was important to underline how the use of Effective microorganisms guaranteed, despite the reduction of irrigation and fertilisation by 50% in the growing medium, the same results in terms of agronomic parameters and fruit production and quality as the control with irrigation and fertilisation under optimal conditions.

In (Table 1), in *Myrtillocactus geometrizans* there was a significant increase in plant height in (EM100) with 38.58 cm compared to 36.34 cm in (EM50), 32.56 cm in (CTRL100) and 26.94 cm in (CTRL50) (Figure 2). In addition, there was a significant increase in plant circumference 9.30 cm in thesis (EM100), 7.82 cm in (EM50), compared to the control at optimum fertilization and irrigation 7.68 cm and 6.70 cm in (CTRL50). Regarding vegetative weight, thesis (EM100) was the best with 50.52 g, followed by (CTRL100) with 46.30 g, (EM50) 44.78 g and (CTRL50) with 38.76 g. The same trend for roots weight where (EM100) showed a weight of 33.84 g, (CTRL100) 30.26 g, (EM50) 28.06 g and (CTRL50) 27.70 g (Figure 3). In terms of flowers number, (EM100) was the best thesis with 21.41, succeeded by (EM50) with 18.80, (CTRL100) with 16.70 and (CTRL50) with 13.40. Also in terms of garambullos production, (EM100) was the improved thesis with 96.60 g, followed by (CTRL100) with 90.50 g, (EM50) 90.08 g and (CTRL50) with 88.02 g. The trial also showed a significant increase in the number of plant thorns 37.00 in (EM100), 33.20 (CTRL100), 32.80 (EM50), 27.60 (CTRL50). Moreover, there was a significant increase in the length of thorns in (EM100) with 2.44 cm and (CTRL100) with 2.26 cm, followed by (EM50) 1.91 cm and (CTRL50) with 1.44 cm (Figure 4).

Table 1 - evaluation of Effective microorganims on the agronomic characters of *Myrtillocactus geometrizans*

Groups	Plant height (cm)	Plant circumference (cm)	Vegetative weight (g)	Roots weight (g)	Flowers number (n°)	Fruit production (g)	Thorns number (n°)	Thorns lenght (cm)
CTRL100	32,56 c	7,68 b	46,30 b	30,26 b	16,70 c	90,50 b	33,20 b	2,26 a
CTRL50	26,94 d	6,70 c	38,76 c	27,70 c	13,40 d	88,02 c	27,60 c	1,44 c
EM100	38,58 a	9,30 a	50,52 a	33,84 a	21,41 a	96,60 a	37,00 a	2,44 a
EM50	36,34 b	7,82 b	44,78 b	28,06 c	18,80 b	90,08 b	32,80 b	1,91 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: (CTRL100): control with 100% water and fertilization; (CTRL50): control with 50% water and fertilization; (EM100): Effective microorganisms with 100% water and fertilization; (EM50): Effective microorganisms with 50% water and fertilization

Table 2 - content of betalains, total phenols and flavonoids and ascorbic acid in plants of *Myrtillocactus geometrizans*

Groups	Betalaines Total (mg/Kg)	Ascorbic acid (mg/Kg)	Fenols total (mg EAG/kg)	Flavonoids Total (mg Eq/kg)
CTRL100	138,68 b	299,58 b	7998,30 a	7477,10 b
CTRL50	134,68 c	289,46 c	7906,26 b	7423,50 c
EM100	143,21 a	304,32 a	8029,26 a	7529,14 a
EM50	135,86 c	298,48 b	7996,54 a	7437,80 c
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: (CTRL100): control with 100% water and fertilization; (CTRL50): control with 50% water and fertilization; (EM100): Effective microorganisms with 100% water and fertilization; (EM50): Effective microorganisms with 50% water and fertilization

The use of Effective Microorganisms also resulted in an increase in the nutraceutical qualities of mature garambullos produced by *Myrtillocactus geometrizans*. In fact, with regard to total betalains, the thesis (EM100) was the best with 143.21 mg/Kg, followed by (CTRL100) with 138.68 mg/Kg, (EM50) with 135.86 mg/Kg and (CTRL50) with 134.68 mg/Kg. Thesis (EM100) was also the best for ascorbic acid production with 304.32 mg/Kg, succeeded by (CTRL100) with 299.58 mg/Kg, (EM50) with 298.48 mg/Kg and 289.46 (CTRL50). There was also significant increase in total phenols in (EM100), (CTRL100) and (EM50) with 8029.26 mg EAG/kg, 7998.30 mg EAG/kg and 7996.54 mg EAG/kg respectively. Finally, thesis (EM100) was also the best for the production of total flavonoids with 7529.14 mg Eq/kg demonstrating that treatment with Effective microorganisms can improve not only plant growth but also the productive and metabolic quality of *Myrtillocactus geometrizans* fruits.

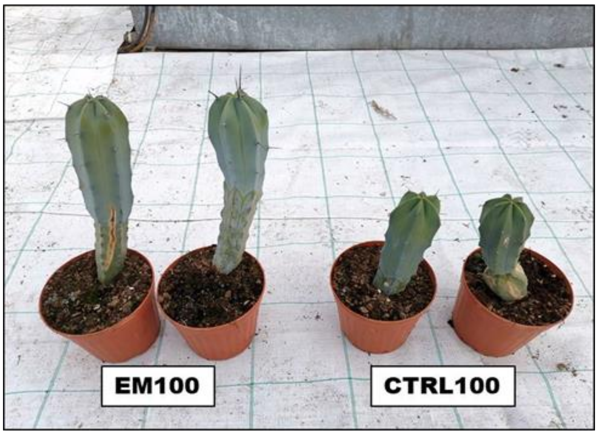

Figure 2 - Comparison between *Effective microorganisms* (EM100) and control with 100% water and fertilization (CTRL100) on growth of *Myrtillocactus geometrizans*

Figure 3 - Effect of Effective microorganisms with 50% water and fertilization (EM100) on roots growth of *Myrtillocactus geometrizans*

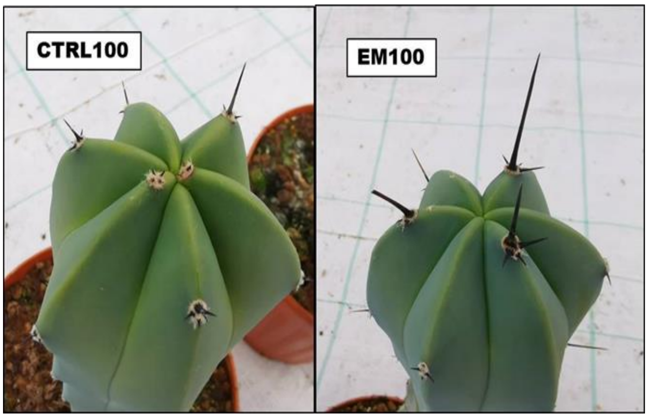

Figure 4 - Effect of *Effective microorganisms with* 100% water and fertilization (EM100) on length of plant thornes of *Myrtillocactus geometrizans*

4. Discussion

Myrtillocactus geometrizans is a high-value agricultural resource that has hardly been exploited and deserves to be exploited industrially. The flowers and fruits are consumed for their high nutritional value, and a homemade alcoholic beverage is produced from the fruits. The stalks are used as a food supplement and fodder for animals, with care taken to remove the areoles and thorns. Similarly, the fruits and the fruit shell are used for the preparation of silage or for fresh consumption by ruminants. The plant is used in endangered areas to protect the soil from erosion, increase organic matter and stabilise sandbanks. It has the property of capturing CO2 as an ideal species to combat climate change, as well as providing food and protection for wildlife. The dried wood is used to make handicraft tools and as firewood for burning. From the dried and ground fruits, pigments of different shades are obtained for dyeing traditional fabrics and garments. The fruits are used to facilitate the fermentation of pulque, a traditional Mexican drink made from maguey. The flowers are used in home cooking cooked as a vegetable or as stew and soup seasonings. In traditional medicine, the healing properties of garambullos are effective in treating diabetes, ulcers and certain types of cancer [4].

Various rhizobacteria are able to promote plant growth through increased root development and increased nutrient uptake. A well-known example is the free-living N2-fixing Azospirillum, which can fertilise wheat, sorghum and maize mainly by increasing root development and thus increasing the rate of water and mineral uptake [23]. Some bacteria are able to increase plant growth by solubilising phosphate from bound organic or inorganic phosphates [24, 25]. Some bacteria produce substances that stimulate plant growth, in particular auxins and other hormones, as well as some volatiles and the cofactor pyrrolquinoline quinone (PQQ). Some rhizobacteria, such as strains of *B. subtilis*, *B. amyloliquefaciens* and *Enterobacter cloacae*, promote plant growth by releasing volatiles [26]. The literature does not reveal studies on the effects of Effective microorganisms on the quality of *Myrtillocactus geometrizans* and in particular in cactus plants, although several works show the effects of this microbial selection on horticultural, ornamental and fruit crops [27,28,29,30,31,32].

In this test, plants treated with (EM) showed a significant increase in plant height and circumference, vegetative and root weight, flower and fruit production, number and length of thorns. In addition, there was also an improvement in nutraceutical parameters of mature garambullos in particular total betalains, ascorbic acid, phenols and total flavonoids. This improvement in plant and fruit quality was also observed in theses treated with Effective microorganisms and a 50% reduction in fertiliser and irrigation water. This aspect was probably determined by the stimulation of root growth, the efficiency of nutrient assimilation by the plant and mediated by the Effective microorganisms, and a greater solubility of the mineral elements present in the growing medium. It is also known how microorganisms can improve plant resistance to abiotic stresses, particularly water stress

5. Conclusion

The test showed how the use of Effective microorganisms can improve the quality and growth of fruit cacti like *Myrtillocactus geometrizans*. In particular, the use of these microorganisms resulted in a significant increase in plant height and circumference, vegetative and root weight, number of flowers and garambullos (fruit) production and thornes number and length. These aspects were also found in previous trials in other vegetables and ornamental crops, but not in fruit cacti. The use of Effective microorganisms also led to an increase in the content of useful metabolites in *Myrtillocactus geometrizans* fruits, in particular betalains, ascorbic acid, phenols and flavonoids, demonstrating how the use of certain microbial strains can metabolically influence plant growth and production. Another aspect confirmed in this experiment was the possibility of cultivating plants by reducing the amount of fertiliser and irrigation turnover, while still ensuring excellent production quality. The application of Effective Microorganisms in agricultural processing can therefore guarantee higher production standards, with a possible reduction in costs fertiliser and water. Particularly for those farms that want to focus on the production of ornamental and fruit cacti. Fruits obtained from growing plants treated with Effective microorganisms have a high antioxidant and nutraceutical potential, which is very important especially in this age where food is also a medicine.

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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