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Structured water uses in improving rhizosphere microbiota and growth of *Cleistocactus strausii*

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Abstract

Research objective: The paper presents the results of research aiming plant growth and to stimulate microbial communities in rhizosphere of *Cleistocactus strausii* which was watered with structured water under controlled conditions.

Materials and Methods: The experiments, which began in January 2023, were conducted in the CREA-OF greenhouses in Pescia (PT), Tuscany, Italy (43°54′N 10°41′E) on *Cleistocactus strausii*. The plants were placed in ø12 cm pots; 30 plants per thesis, divided into 3 replicates of 10 plants each. The experimental groups were: i) group without structured water, irrigated with water and previously fertilised substrate; ii) group with structured water, previously fertilised substrate. On 20 January 2024, plant height, stem circumference, vegetative weight, root weight, root lenght, number of new suckers, microbial count in the plants (evaluation of the type of bacteria and fungi in the substrate) were measured.

Results and Discussion: The experiment showed that the use of structured water can improve the vegetative and root growth of *Cleistocactus strausii* plants grown in pots. The treatment also resulted in an increase in plant height, stem circumference, root hair length and number of new suckers. The trial also highlighted how the use of structured water in plant growing media can increase bacterial and fungal biodiversity in terms of number and taxonomic diversity. In fact, the substrates of the treated plants showed the presence of 5 different bacterial and fungal taxa compared to the control. The presence of fewer plants affected by biotic phytopathologies is evident in the treatment with structured water.

Conclusions: Specifically, structured water has been shown to promote the growth of *Cleistocactus strausii*, according to experimental results. Plant height, vegetative and root biomass were significantly increased. Additionally, the number of useful microorganisms found on the substrate of theses treated with structured water increased, which likely supported plant growth improvement. Plants can also be affected by microorganisms in their substrates if they are exposed to biotic or abiotic stress. These aspects consequently become very interesting for the grower who can reduce the use of water and fertilisers and increase the quality of the plants by using alternative techniques.

Keywords: Structured water; Sustainable applications; Microorganisms; Rhizosphere; Hydrophilic

1. Introduction

The physicochemical properties of structured water include increased electrical conductivity and pH [1]. It is a semicrystalline form of water with a higher viscosity, lower density, and surface tension. As the ratio between structured and unstructured water increases, water conductivity and pH increase because more electrons and protons form vortices in and around the hexagonal rings of water, which are delocalized and quasi-free. A structured water can

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also be referred to as biological water, bound water, activated water, energized water, coherent domain water, vitalized water, or hexagonal water [2]. When unstructured, liquid water is exposed to a combination of chemical and/or electromagnetic energy sources, such as ozone or hydrogen peroxide combined with ultraviolet light or magnetic fields, a fraction of the water molecules will decompose into hydroxyl radicals. Water treatment systems based on the hydroxyl generator technology are available, which is a combination of ultraviolet lamps with wavelengths of 185 nm or shorter. In addition to the strength of magnetic fields, the mineral composition of the water and its temperature influence the ratio of structured to bulk water [3]. Many agricultural applications benefit from structured water because it is free from energetic toxins. Besides increasing energy, it also regulates and balances soil minerals, and it brings forth a high oxygenation state. Structured water helped strawberries, tangerines, sprouts, lemon and grapes grow faster and healthier, mature sooner, yield more delicious food, and keep it fresher longer (shelf life) [4]. Generally, structured water gains the following benefits: 100% increase in fruit, grain, vegetable production; 60% reduction in water usage; 100% reduction in chemical usage; better pest, mould, algae control; healthier crops, birds, cows; resistance to extreme temperatures; improved soil conditions; increased flavor, texture, and shelf life. A study of the antioxidant properties of structured water and its effect on animal cell bioactivities revealed that it helps normal cells while suppressing malignant cells, which is good for both animals and humans [5]. In terms of structured water, Prof. Gerald Pollack of the University of Washington is a pioneer, as he defined the fourth phase of water, also referred to as structured water. A hexagonal structure can be observed with nuclear magnetic resonance spectroscopy (NMR), which has been the subject of several scientific publications in research journals. A higher yield in plants results from increased hydration of the cell walls. As a result, structured water is highly applicable to agriculture [6]. Due to its high density compared to ordinary water, suspended microspheres are excluded from suspended water, resulting in the exclusion zone, which has been named as such. Additionally, it has been observed that an electrical potential of -200 mV develops outside the exclusion zone and beyond its boundaries (negative exclusion zone) [7].

The paper presents the results of research aiming plant growth and to stimulate microbial communities in rhizosphere of *Cleistocactus strausii* which was watered with structured water under controlled conditions.

Figure 1 Details of the *Cleistocactus strausii* plants used in the trial

2. Material and methods

The experiments, which began in January 2023, were conducted in the CREA-OF greenhouses in Pescia (PT), Tuscany, Italy (43°54′N 10°41′E) on *Cleistocactus strausii*. The plants were placed in ø12 cm pots; 30 plants per thesis, divided into 3 replicates of 10 plants each. All plants were fertilised with a controlled-release fertiliser (2 kg m^{-3} Osmocote Pro®, 6 months with 190 g/kg N, 39 g/kg P, 83 g/kg K)mixed into the growing medium before transplanting.

The experimental groups were:

- Group without structured water (CT) (peat 70% + pumice 30%), irrigated with water and previously fertilised substrate;
- Group with structured water (SW) (peat 70% + pumice 30%), previously fertilised substrate (5 ml per plant every day).

The Alchewat alpine fountain was used to structure the water, which, according to the manufacturer, vitalises and improves the water through biomagnetism. According to the manufacturer, this device vitalises and improves water through biomagnetic induction processes and with the help of transmaterial catalysts. The device transmits a structured modulating frequency to the water flowing through the tube. This changes the molecular behaviour of the water molecular behaviour of the water and brings it to a level similar to that of pure spring water. The device sends a modulating structured frequency to the water flowing through the pipe. This changes the molecular behaviour of the water and brings it to a level similar to pure spring water. The device is mounted on the irrigation pipe and consists of a mixture of minerals, precious metal alloys diamagnetic transition metals and alpine spring water. The entire water restructuring process takes place in an absolutely ecological manner, requiring no maintenance or chemicals and no electricity. The plants were watered twice a day and grew for 14 months. The plants were drip-irrigated.The irrigation was activated by a timer whose programme was adjusted weekly according to the weather conditions and the leaching fraction. On 20 January 2024, plant height, stem circumference, vegetative weight, root weight, root lenght, number of new suckers, microbial count in the plants (evaluation of the type of bacteria and fungi in the substrate) were measured.

2.1. Microbiological analysis of substrate samples

Microbiological analysis of the substrate samples was performed by plating decimal dilutions of soil on specific solid culture media Nutrient Agar (NA) for aerobic heterotrophic bacteria and Czapek for fungi. After incubation in the dark, the microbial colonies developed were counted and the values reported in grams of dry soil. Taxonomic identification of the bacteria was carried out according to Bergey's manual of determinative bacteriology [8]. Fungi were identified according to the determinative manuals of Domsch and Gams (1970) [9] and Watanabe (2002) [10]. All tests were performed in triplicate.

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 and discussion

The experiment showed that the use of structured water can improve the vegetative and root growth of *Cleistocactus strausii* plants grown in pots (Figure 3 and Figure 4). The treatment also resulted in an increase in plant height, stem circumference, root hair length and number of new suckers (Table 1).

The trial also highlighted how the use of structured water in plant growing media can increase bacterial and fungal biodiversity in terms of number and taxonomic diversity (Figure 2 and Table 2). In fact, the substrates of the treated plants showed the presence of 5 different bacterial and fungal taxa compared to the control. The presence of fewer plants affected by biotic phytopathologies is evident in the treatment with structured water.

The molecular arrangement of water molecules in structured water is formed when water is in contact with hydrophilic surfaces (water loving) [11]. The water molecules unite in monolayer sheets with a hexagonal structure, growing the layers, while the protons are ejected into the adjacent water [12]. The structure of water in general has a number of properties, such as: i) inhibition of dissolved substances (such as limestone) from deposition or fouling, which can be very beneficial for boilers and connecting pipes to be free of fouling; ii) low surface tension and increased wetting properties; iii) pH is slightly above 7, which allows it to be assimilated into body fluids; iv) neutralizes the effect of acid chlorine; v) 'structured water strengthens the hydrogen bond [13-17].

In agriculture, structured water can provide the following advantages: i) increased soil health with improved plant growth; ii) increased soil efficiency in supplying plants with nutrients; iii) increased plant capacity to absorb nutrients from the soil; iv) increased soil water retention with reduced irrigation turnover (20-50%); v) improved plant quality and shelf-life; vi) increased vegetative quality of plants and reduction of diseases; vii) increased crop quality and quantity; viii) improved flower colour intensity; ix) increased antioxidant properties of water. Several cellular molecules, including water, may be active in cell biology. Using structured water can result in increased yields in winter wheat (28%), cucumber (32%), and tomato (32%) [18-21]. It can also improve milk production and fertility in cattle, meat and egg production in poultry, both qualitatively and quantitatively. In line with literature and field observation data, structured water has a beneficial effect on plant growth, health, yields, and quantity [22]. The use of structured water on lavender and rosemary actually promotes plant growth, vegetative and root development, total flower biomass, reducing dried plants and an interesting aspect is that the microbial count of treated substrates increases.

According to experiments conducted by private and public farmers in America, it is possible to reduce water consumption by 20-30% as well as increase shelf life and nutrient density while cultivating important fruit species [23-24].

The results in the experiment are in concordance with data from literature that report a beneficial effect of structured water on basil, cucumber, tomato and strawberry plants growth, health, yields quantity and quality as compared to plants watered with tap water [24].

Table 1 Evaluation of Structured water on agronomic characters on plants of *Cleistocactus strausii*

Cleistocactus strausii	PH (cm)	SC (cm)	VW (g)	RW (g)	RL (cm)	NS (n°)
CT	37.98 b	5.89 b	42.27 b	20.21 b	6.32 b	0.20 b
SW	44.00 a	7.01 a	49.56 a	24.68 a	7.56 a	1.60 a
ANOVA	***	**	***	***	***	**

One-way ANOVA; n.s. – non significant; *,**,*** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$). Parameters: PH = plant height (cm); SC = stem circumference (cm); VW = vegetative weight (g); RW = roots weight (g); RL = roots length (cm); NS= new suckers. Treatments: CT=control; SW=structured water

Table 2 Taxonomic composition of bacterial and fungal microflora

Experimental group	Bacterial microflora Taxonomic composition	Fungal microflora Taxonomic composition
CT	Pseudomonas fluorescens, Bacillus cereus, Bacillus megaterium, Micrococcus sp.	Trichoderma viride, Aspergillus sp.
SW	Pseudomonas fluorescens, Bacillus cereus, Bacillus circulans, Pseudomonas acidophila, Arthrobacter sp., Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus sp, Bacillus mesentericus	Trichoderma viride, Trichoderma harzianum, Aspergillus sp., Fusarium oxysporum, Penicillium sp., Acremonium strictum, Paecilomyces chrysogenum

Treatments: CT=control; SW=structured water

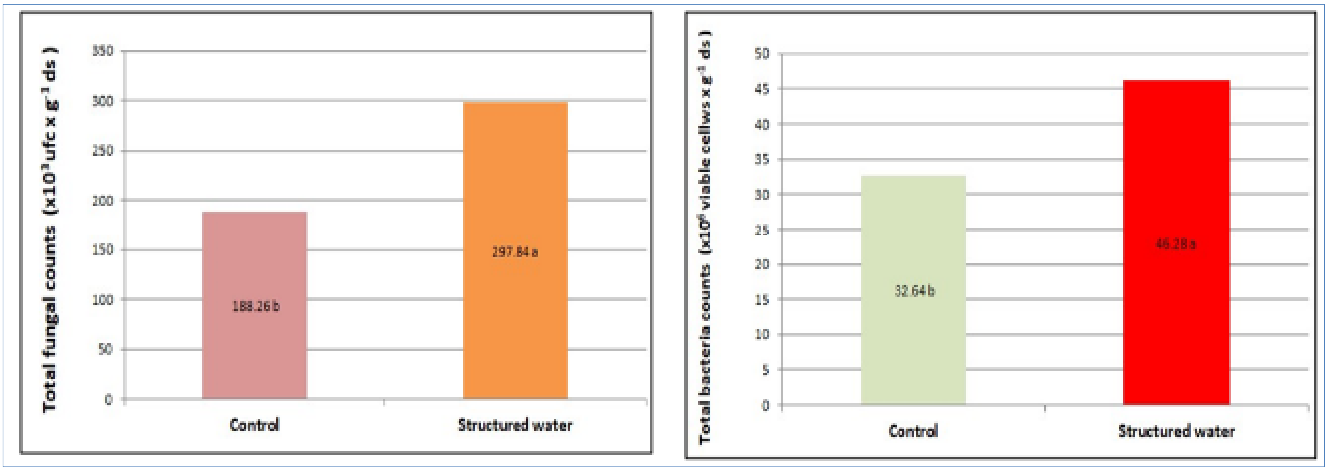

Figure 2 Influence of structured water on fungal and bacteria counts

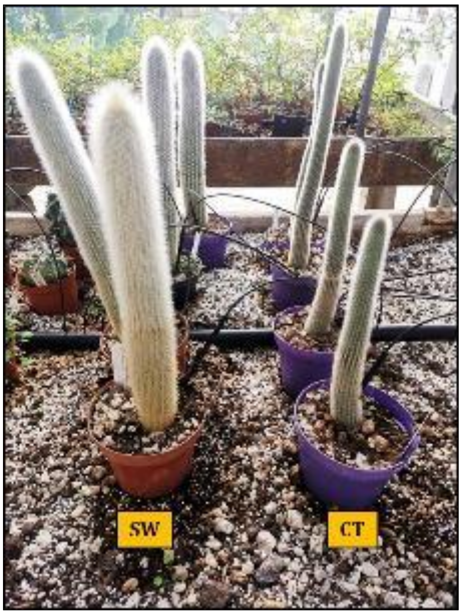

Figure 3 Effect of structured water on vegetative biomass, plant height and stem circumference of *Cleistocactus strausii*. Legend: (CT): control; (SW): structured water

Figure 4 Effect of structured water on root biomass and root hair size. Legend: (CT) control; (SW): structured water

4. Conclusion

Specifically, structured water has been shown to promote the growth of *Cleistocactus strausii*, according to experimental results. Plant height, vegetative and root biomass were significantly increased. Additionally, the number of useful microorganisms found on the substrate of theses treated with structured water increased, which likely supported plant growth improvement. Plants can also be affected by microorganisms in their substrates if they are exposed to biotic or abiotic stress. These aspects consequently become very interesting for the grower who can reduce the use of water and fertilisers and increase the quality of the plants by using alternative techniques. *Cleistocactus* plants accumulated significantly higher total biomass and greater root surface area when irrigated with structured water compared to the control. Structured water stimulated the development of heterotrophic bacterial communities, dominated by *Pseudomonas fluorescens*, and species diversity increased by 5 taxa. Structured water also improved the structure of fungal communities, decreased colonisation with potentially plant pathogenic species, due to the dominance of antagonistic *Trichoderma* and *Paecilomyces* species. The results recommend structured water to enhance the growth of certain cactus species and the rebalancing of the rhizosphere microbiota.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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