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THE INFLUENCE OF QUANTUM STRUCTURED WATER ON MICROBIAL DIVERSITY IN RHIZOSPHERE OF TOMATO AND ASSESSMENT OF BIOLOGICAL QUALITY OF PLANTS BY IMAGE-FORMING METHODS

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Abstract

The structured water is obtained by quantum technology, exclusively on the basis of natural elements, under the influence of electromagnetic field with magnitude of the waves in the order of 10^{-40}. The aim of this paper is to present the results of research carried out in greenhouse conditions to assess the influence of irrigation with quantum structured water, as compared with irrigation with tap water, on biodiversity and structure of microbial communities in rhizosphere of tomato (Solanum lycopersicum L.*) FLAVIOLA variety and to evaluate the biological quality of plants. The paper presents the total counts and species of bacteria and fungi estimated by dilution plate method, diversity index of Shannon (H), equitability and similarity index. The influence of structured water on plants hight, fresh biomass accumulated, biological quality and vitality are discussed comparatively with tap water using image-forming methods, namely biocrystallization, circular chromatography and capillary dynamolysis by the evaluation of structures formed consequently to the reaction of plant extracts with certain inorganic salts.*

Key words: *tomato, biodiversity, microbial communities, quantum structured water, image-forming methods.*

INTRODUCTION

Over the recent years, water science has developed enormously in diverse disciplines including physics, chemistry and biology, opening broad perspectives for new tehnological applications, due to the better understanding of its properties.

A comprehensive review (Kontogeorgis et al., 2022) synthesized important information and discussions about water structure, properties and some applications significant for engineering, chemistry, biology and medicine.

Some of novel experiments, theories and inventions refer to the study of "structured water" and the benefits from its applications (Germano, 2015; Dubey et al., 2018).

Recent studies reported the beneficial effect of irrigation with structured water on plants growth and health, yields quality in horticultural plants of tomato, cucumbers, basil, strawberry (Abraham, 2014; Enache et al., 2019a).

A series of picture forming methods are widely utilized in EU countries for acquiring images reflecting the vitality of plants, biological quality assessment of fresh foods, medicaments from medicinal plants, for certification in biodynamic agriculture or differentiation between the efficiency of various agricultural practices.

Thus, circular paper chromatography, capillary dynamolysis or sensitive crystallization as holistic methods, complementary to other methods of quality monitoring, helped successfully in discriminating between various produces from organic and conventional origin (Weibel et al., 2000; Szulk et al., 2010; Fritz et al., 2011, 2020).

A less studied aspect was the application of image-forming method to the assessment of plant quality and vitality under irrigation systems using different types of water.

The aim of the present research was to assess the influence of irrigation with quantum structured water, as compared with irrigation with tap water, on biodiversity and structure of microbial communities in rhizosphere of tomato *(Solanum lycopersicum* L.*)* and to evaluate the biological quality of plants by image forming methods.

MATERIALS AND METHODS

Structured water. The quantum technology, created exclusively on the basis of natural elements was applied to obtain structured water. Tap water was introduced into the apparatus with quantum electromagnetic field with magnitude of the waves in the order of 10^{-40} to obtain structured water. During the water structuring process, the information transported by this electromagnetic field remains captive (stored) in the aqueous physical vacuum from the coherence fields of the water and it is rewritten through the water into the body of the plant.

Experimental variants. The experiment was carried out under controlled conditions of humidity and temperatures (24ºC days and 22ºC night) in Hortinvest greenhouse of the Research Center for Horticultural Products Quality, Faculty of horticulture, UASVM Bucharest, during March-June 2020.

Tomato plants (*Solanum lycopersicum* L.) Flaviola variety with indeterminate vegetative growing and fruit cherry type were watered with quantum structured water (V1). Similar pots were prepared and the tomato plants were watered with tap water (Control). The dose of nitrogen, phosphorus and potassium was the same for both variants.

Plants height (cm) was measured and **total biomass** (grams) **accumulated** was determined by weighing tomato plants from experimental variants.

Microbiological analyses were performed accordingly to soil serial dilution method using specific culture media with agar-agar: Nutrient agar (NA) for aerobic heterotrophic bacteria and potato-dextrose agar (PDA) for fungi. Microbial density was expressed as Total Number of Bacteria-TNB and Total Number of Fungi-TNF reported to gram of dry soil (Dumitru & Manea, 2011).

Taxonomic identification of microbial species in rhizosphere of tomato plants was done using morphologic criteria, according to the determinative manuals (Bergey & Holt, 1994) for heterotrophic bacteria and Domsch & Gams (1970) for fungi. Morphological features were examined and photographed under a MC 5.A optic microscope.

The relative abundance of each species in the structure of microbial community was calculated. Species richness (SR_2 index) in microbial communities from tomato rhizosphere was calculated as the ratio between the total number of species (S) and microbial counts from each experimental variant.

The Shannon index (H') that takes into account both the richness and evenness (ε) of a community was calculated for evaluating the microbial biodiversity in rhizosphere of tomatoes in variant with quantum structured water, as well as in control (tap water) (Mohan & Ardelean, 1993).

The increased value of H' reflects the increased number of species and also the evenly distribution of the species (Morris et al., 2014).

The "equitability" as component of microbial species diversity (distribution of "individuals" on species) was calculated according to Stugren (1982).

Similarity indices (SI) were calculated (Tiwari et al., 1994) for comparative analysis of the microbiome composition.

Picture forming methods used for acquiring new images reflecting the vitality and biological quality of tomato plants were represented by circular paper chromatography, capillary dynamolysis and sensitive crystallization.

Capillary dynamolysis: The differences of biological quality between tomato plants under the influence of experimental conditions of irrigation with quantum structured water and tap water were evaluated by the capillary rising picture method called capillary dynamolysis, according to Kolisko (1953) and refined by Zalecka et al. (2010). A standard quantity of aqueous filtered tomato leaf extract was migrated on a vertical tube of filter paper in a Kaehlin glass dish, followed by intermediate drying, by metal salt (silver nitrate) and iron sulphate migration and development of specific images.

Images of capillary dynamolysis obtained after the development of colors were scanned for analysis of structures using the criteria described by Zalecka et al. (2010), Unluturk et al. (2011) and Böttgenbach (2018).

Sensitive crystallization was another alternative method, setup by Pfeiffer (1984), we used for evaluating the vitality and biological quality of tomato under the influence of tap or quantum structured water by analysis of images of copper chloride crystals formed in contact with leaf extracts to observe the modification of aspect and morphology of the crystallization network.

Circular paper chromatograms were made by migration of tomato leaf extracts through a circular filter paper, previously impregnated by developing substance, to obtain information on biological quality of test plants, according to procedure and recommendations of Pfeiffer (1984).

RESULTS AND DISCUSSIONS

The results of microbiological analyses on total counts of bacteria, fungi and values of indices that characterize biodiversity of each microbial community from rhizosphere of tomato plants from Control and from the variant watered with quantum structured water are presented in Table 1.

Table 1. Total counts and biodiversity indices of bacterial and fungal microflora in rhizosphere of tomato watered with quantum structured water (V1) as compared with tap water (control)

Experimental variant	Bacterial microflora	Fungal microflora
CONTROL (watered with tap water)	TNB=15 x 10^6 viable cells x g^{-1} d.s. S=6 SR_2=0.400 H'=1.714 ε=0.724	TNF=23 x 10^3 cfu x g^{-1} d.s. S=4 SR_2=0.173 H'=1.338 ε=0.695
Variant V1 (watered with quantum structured water)	TNB=12 x 10^6 viable cells x g^{-1} d.s. S=7 SR_2=0.583 H'=1.792 ε=0.701	TNF=15 x 10^3 cfu x g^{-1} d.s. S=7 SR_2=0.466 H'=1.626 ε=0.615
	SI=61.54%	SI=36.36%

Data evidenced moderate densities of bacteria (15 x 10^6 viable cells x g^{-1} d.s. for control and 12 x 10^6 viable cells x g^{-1} d.s. for variant with quantum structured water) and low levels of fungal counts (23 x 10^3 cfu x g^{-1} d.s. for control and 15 x 10^3 cfu x g^{-1} d.s. for variant with quantum structured water).

Microbial diversity was higher in both bacterial and fungal communities from rhizosphere of tomato plants watered with quantum structured water as compared with values of Shannon Diversity Index from control. Thus, a higher number of bacteria species (7) with H'=1.792 and less homogeneous distribution (ε=0.701) were recorded in variant with quantum structured water as compared with control (H'=1.714) but more homogeneous distribution of effectives between the 6 species (ε=0.734).

Fungal community from variant with quantum structured water was more diversified, with 7 species, H'=1.626 and lower homogeneity ε=0.615 as compared with control (4 species, H'=1.338) and more homogeneous distribution (ε=0.695).

Taxonomic composition and the relative abundance values of the bacteria species revealed that the fluorescent pseudomonads were the most abundant in tomato rhizosphere from both control (Figure 1) and the variant with quantum structured water (Figure 2), with A=26.7% and 33.3%, respectively, followed by various species of *Bacillus* and actinomycetes from Series Albus, with lower abundance in population.

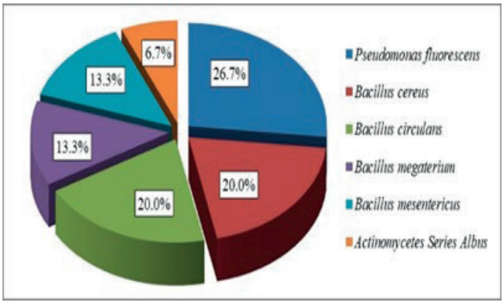

Figure 1. Percent mean relative abundance of bacterial microflora composition in tomato rhizosphere at control (tap water)

The rhizosphere community of fungi isolated from control (Figure 3) was characterized by the dominance of *Fusarium oxysporum* (A=34.8%) and *Penicillium* spp. (A=30.4%), accompanied by less abundant *Aspergillus ochraceus* and *Trichoderma viride* (A=17.4%, each).

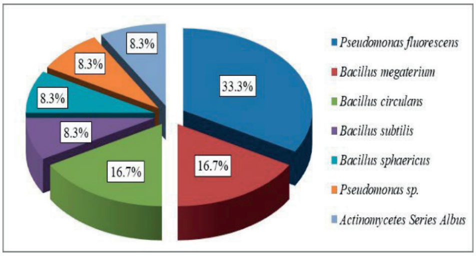

Figure 2. Percent mean relative abundance of bacterial microflora composition in tomato rhizosphere at variant with quantum structured water

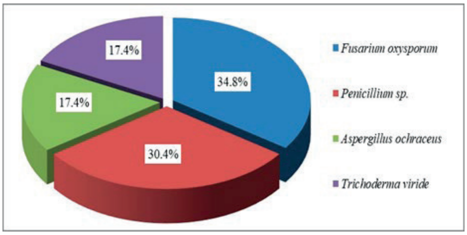

Figure 3. Percent mean relative abundance of fungal microflora composition in tomato rhizosphere at control (tap water)

Watering with quantum structured water increased the biodiversity by stimulating the development of new species of the genus *Penicillium* (e. g. *Penicillium funiculosum,* dominant with A=26.7%, *Penicillium variabile* with A=20%) and proliferation of *Trichoderma viride* with A=20%, higher than in the mycocoenosis from control. Other new species but less abundant in tomato rhizosphere belonged to the genera *Cladosporium, Mortierella, Verticillium* (Figure 4).

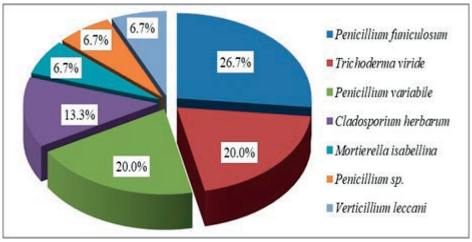

Figure 4. Percent mean relative abundance of fungal microflora composition in tomato rhizosphere at variant with quantum structured water

Similarity Index value SI=61.54% calculated between taxonomic composition of bacterial communities revealed that more than half of species identified were present in both rhizosphere communities. The shared species were *Pseudomonas fluorescens, Bacillus megaterium, Bacillus circulans* and Actinomycetes from Series Albus.

Fungal communities were more different, with a Similarity Index value SI=36.36% reflecting a particular composition and structure and fewer shared species, represented by *Trichoderma viride* and *Penicillium* sp.

In the rhizosphere of plants, soil particles are bound together by phenolics and other exudate compounds released by roots, microorganisms and decomposing organic matter, forming aggregates, thus providing support for plant roots (Makoil & Ndakidemi, 2007).

The majority of microbial species identified in rhizosphere of tomato from our experiment are recognized in literature for the property to perceive signals secreted from the plant root and also to release different signaling molecules for the control of host plant plant growth, root development, as well as for improving biotic and abiotic stress tolerance or eliciting mechanisms of resistance to various pathogens (Weller et al., 2002; Zhang et al, 2017; Yan et al., 2021).

Thus, the results revealing the presence of beneficial species (e. g. fluorescent or non-fluorescent pseudomonads, *Bacillus subtilis*, *Bacillus megaterium*, actinomycetes, *Trichoderma viride,* species from genera *Penicillium, Mortierella*) in tomato rhizosphere are in concordance with research carried out by Jaiswal et al. (2017) who found a link between microflora biodiversity from tomato rhizosphere and suppressiveness effect against various soil-borne pathogens.

Redouan et al. (2018) reported the potential production of volatile and diffusible antifungal metabolites by selected strains belonging to genus *Pseudomonas*, against a wide range of phytopathogenic fungi that infect tomato plants.

Trichoderma spp. are known from literature for antimicrobial properties against numerous phytopathogens and for a complex enzymatic equipment with role in organic matter recycling (Kucuc & Kivanc, 2003; Gurău et al., 2021; Pani et al., 2021).

Literature data estimated that about 2–5% of bacteria from rhizosphere have plant-growth-promoting traits and are important as potential tools for sustainable agriculture in the future (Alawiy & Babalola, 2019).

Various methods have been developed for improving plants growth, yield, resistance to pathogens or quality. Grafting was reported as a practice meant to increase productivity, or to improve some qualities of tomato fruits such as the soluble dry substance content, the total amount of carbohydrates and vitamin C (Sora et al., 2019).

The influence of quantum structured water was reflected by increased plant hight (138 cm) and total biomass accumulated (991 grams) as compared with the control (123 cm and 916 grams), as presented in Table 2.

Table 2. Plant hight and total biomass accumulated

Experimental variant	Plant height (cm)	Total biomass (grams)
CONTROL (watered with tap water)	123	916
Variant V1 (watered with quantum structured water)	138	991

In 'Flaviola' variety, the sum of the cumulative temperatures and CO_2 had a great influence on the production and quality of tomato fruits (Jerca et al., 2021).

Research results (Jerca et al., 2016) evidenced the influence of watering rate and the type of substrate on the production of tomatoes grown in the greenhouse in unconventional system.

Additional picture (image) forming methods utilized provided new evidences for evaluation of tomato plants quality in control and under the influence of quantum structured water.

Visual evaluation of the paper images formed by **capillary dynamolysis** was carried out evidencing few differences in the aspect of structural elements from base, bowl and flag zone. In both pictures, the three layers of the basal zone are well defined but the colors were more intense at the variant with quantum structured water than in image from the sample watered with tap water.

In control (Figure 5), corona is more irregular, sometimes interrupted by white beards, short flags start from the base and rise not parallel, in apparent disorder, occupying only 1/3 from this zone, ended as thin lines. The apical 2/3 of flag zone are diffuse and the large white zone ended with a thick brown layer, with irregular contour without reduction spots.

In the picture from the variant with quantum structured water (Figure 6), corona is more uniform, without beards. Bowl zone is more diffuse in forms and more colored and continues with a high flag zone containing parallel pipes narrowed towards the drop zone, rarely started from the base, continued in a diffuse grey zone and more often formed on the apical third in the image from the sample watered with structured water. Open ended long pipe in the center of image passes the thin white zone, being in contact with long brown reduction spots coming in single or sometimes in pairs from the thin superior irregular layer.

Figure 5. Capillary dynamolysis of tomato in control watered with tap water

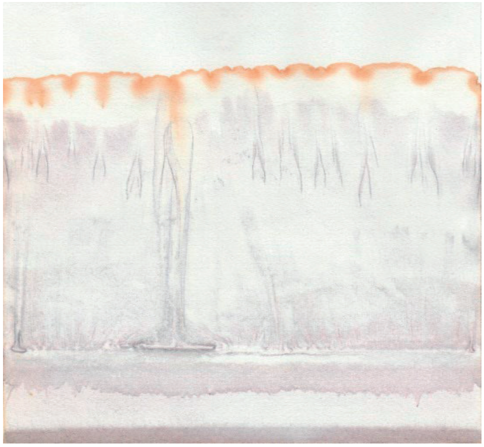

Figure 6. Capillary dynamolysis of tomato in variant watered with quantum structured water

Previous study (Enache et al., 2019b) revealed that irrigation with structured water significantly increased plant height and fresh biomass accumulation as compared to tap water and presented capillary dynamolysis images reflecting the vitality and biological quality of cucumber plants. As in the present experiment, a higher vital force and quality of plants by stronger, more intensely colored and better-defined images were obtained at variants with structured water, more pronounced when added supplementary biological or chemical fertilizers.

Sensitive crystallization generated optimally integrated whole pictures in both control (Figure 7) and in the variant with quantum structured water (Figure 8), showing a center zone located 2-3 cm away from the geometrical center from which bundles of branches and needles spread towards the margins of the Petri plates.

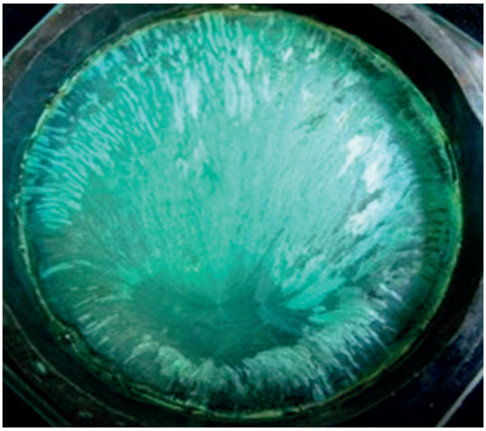

Figure 7. Sensitive crystallization of tomato in control watered with tap water

The two-centered branches and needles from the middle zone are thicker than those from the peripheral zone and more thickened in the variant with quantum structured water than in control. In both crystallization pictures appear numerous curved branches and needles more clearly evidenced in Figure 8.
Similarly, results of Popovic-Vrajes et al. (2016) confirmed that biocrystallisation patterns obtained using crystallisation method, (as a holistic method utilised in order to emphasize the benefits of organic food) evidenced the differences between pasteurized organic and conventional milk.

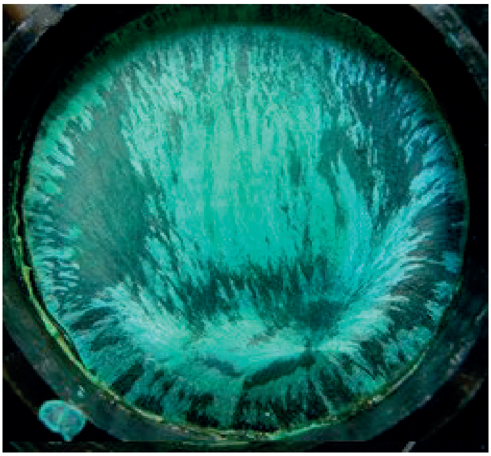

Figure 8. Sensitive crystallization of tomato in variant watered with quantum structured water

Circular chromatograms presented differences of color, general aspect, dimensions of central, intern and especially intermediate zones, as illustrated in Figure 9.

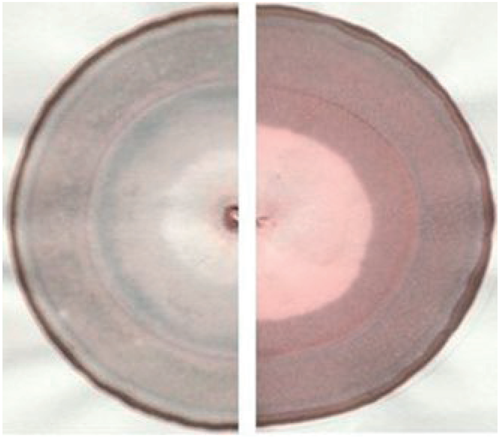

Figure 9. Sections of circular chromatograms of tomato in control (left) and in variant watered with quantum structured water (right)

The chromatogram of tomato plants from the variant with quantum structured water (right) presented lighter colours in reddish brown nuances, better defined forms as compared with more diffuse aspect with blue-grey nuances from control (left).
Our results are in concordance with recent data from literature showing that the three image-forming methods proved to be useful tools for assessing the ageing in terms of degradation (oxidation) of wine from different cultivation systems, completing the sensory analysis (Fritz et al., 2017; 2021).

CONCLUSIONS

Irrigation with structured water significantly increased plant height, fresh biomass accumulation and microbial biodiversity in rhizosphere of tomato plants as compared to tap water.
Differences in the aspect of images obtained by circular paper chromatography, capillary dynamolysis and sensitive crystallization were observed, evidencing a higher vital force and quality of plants watered with quantum structured water.
Information derived from rising pictures analysis proved its usefulness in comparing the

influence of irrigation water on quality of tomato plants.

Further investigations are necessary to link image forming results with usual quality tests for better elucidating significance of differences obtained between control and variant with quantum structured water.

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