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STIMULATION OF PLANT GROWTH AND RHIZOSPHERE MICROBIAL COMMUNITIES BY TREATMENTS WITH STRUCTURED WATER

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Abstract

The purpose of the present research was to improve plants growth and to stimulate microbial communities in rhizosphere of three test plants (cucumber, basil and tomatoes) by watering with structured water using various dilutions in experiments under controlled conditions. Significantly increased bacterial counts and species number registered for all test plant species under the influence of structured water integral and structured water dilution no.10 as compared to control. The highest number of species (12) identified in rhizosphere of cucumber from structured water dilution no. 10 dominated by pseudomonads (Pseudomonas lemonnieri indicating improved qualitative conditions) and bacillaceae. High fungal counts from control and variant structured water dilution no. 3 indicated less favourable conditions for communities where potential plant pathogen Fusarium developed. Trichoderma and Paecilomyces (antagonists for Fusarium) as well as other cellulolytic fungi active in rhizosphere were stimulated in variants with AVVA integral and structured water dilution no.10 best dilution. Significantly higher plants and total biomass accumulation registered for treatments with structured water (structured water integral and diluted variant structured water dilution no. 10) comparatively with control, especially for basil.

Key words*: structured water, soil microorganisms, fungi, bacteria, plant growth stimulation.*

INTRODUCTION

At present there are research indicating the stimulative effect of structured water on plants growth. Thus, the administration of structured water stimulated the growth of brown chickpea seeds germination and showed 2-3-fold increase in root length and shoots formation (Sharma et al., 2017).
Producers of structured Water Units recommend the use of structured water for many agricultural applications because it contains no energetic toxins. It brings forward with high oxygenation state, increases the energy, regulates and balances the soil minerals. Through the use of structured water various crops such as strawberry, tangerine, sprouts, lemon and grapes grew faster and healthy, plants matured sooner, more tasty yields, and the time of staying fresh (shelf life) increased. Generally, benefits gained from using structured water include: up to 100% increase fruit/grain/vegetables; up to 60% reduction in water usage; up to 100% reduction of chemical use; improves pest, mould, algae control; healthier crops, birds, cows; resistance to extreme temperatures; improves soil conditions; enhances taste, texture and shelf life of fruits and vegetables (Sharma et al., 2017).
Research carried out in University of Colorado gained new aspects of obtaining and practical use of structured water technology in large scale agriculture as a further promise for water conservation and increased yields and its quality (Ptok, 2014).
Studies of antioxidant properties of structured water and its effect on animal cell bioactivities (Higgins et al., 2006) revealed that it helps the activities of normal cells while suppressing those of malignant cells (Hwang et al., 2017), proving beneficial effect on animal and human health, too.
The paper presents the results of research aiming to improve plants growth and to stimulate microbial communities in rhizosphere of three test plants (cucumber, basil, tomatoes)

which were watered with various dilutions of structured water under controlled conditions.

MATERIALS AND METHODS

In order to improve plants growth and to stimulate microbial communities in rhizosphere, three test plants (cucumber, basil and tomatoes) were watered with structured water integral (V2) and using two dilutions (V3 - structured water dilution no. 3. and V4 structured water dilution no. 10) in experiments under controlled conditions.

In this study we used 40 variants of water dilutions, but we selected only two because they were highlighted by higher vegetative growths.

For each species of test-plant was used a pot watered with tap water as control (V1). Microbiological analysis of samples collected from rhizosphere of the three test-plant species were performed by plating soil decimal dilutions on specific solid culture media: Nutrient agar for heterotrophic bacteria and Czapek for fungi (Papacostea, 1976).

After incubation, the developed colonies were counted and the densities of microbial structures were reported to gram of dry soil. Taxonomic identification of bacterial isolates was carried out on the basis of Bergey's Manual (1994).

Fungal isolates were identified according to determinative manuals of Domsch and Gams (1970) and Watanabe (2002). The collected data has been statistically processed and represents the mean of three replicates for each variant.

RESULTS AND DISCUSSIONS

Data analysis from experiment evidenced that structured water exhibited variable plant growth promoting properties and influenced microbiota as a function of plant species involved and the variant utilized (non-diluted or diluted) as compared to control.

The results showed that applying of structured water and dilution 10 increased significantly the values of bacterial counts (Fig. 1) and species number for all the plants compared with low to moderate values from control (watered with tap water).

Bacterial communities were generally dominated by fluorescent and nonfluorescent *Pseudomonas* accompanied by *Bacillaceae*, with a maximum of 12 species identified in rhizosphere of cucumber from V4 with structured water dilution no.10 (Fig. 3).

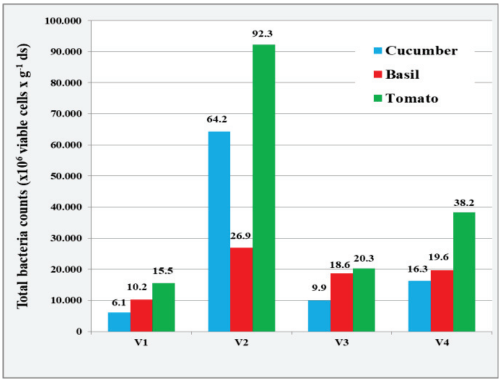

Figure 1. The effect of structured water on bacteria counts

In other variants were identified species connected with humid environments (*Pseudomonas pseudogleyi* in V2, V4 to basil and V4 to cucumber, *Bacillus subtilis* in V1 to cucumber, V2, V4 to basil and V4 to tomato) or *Pseudomonas aeruginosa*, dominant in rhizosphere of basil plants from control (Table 1).

Pseudomonas lemonnieri indicated improved qualitative conditions in variants with structured water integral for cucumber and basil and in the best structured water dilution *no.*10 for variants with cucumber and tomato plants.

Values (Fig. 2) higher than 200 x 10^3 cfu x g^{-1} ds for fungal counts from control (V1) and dilution no. 3. (V3) indicated less favourable conditions for communities where potential plant pathogen *Fusarium* developed as well as two species of the nematophagous genus *Arthrobotrys*. *Trichoderma* (Fig. 4) and *Paecilomyces* (antagonists for *Fusarium*) as well as other cellulolytic fungi active in rhizosphere processes were stimulated in variants with structured water integral and structured water dilution no. 10 (V4) (Table 2).

Table 1. Taxonomic composition of bacterial microflora

Experimental Variant		Bacterial microflora Taxonomic composition
V1	CUCUMBER	*Pseudomonas fluorescens, Pseudomonas aeruginosa, Bacillus megaterium, Bacillus subtilis, Arthrobacter sp., Bacillus circulans, Bacillus sphaericus*
	BASIL	*Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus circulans, Bacillus megaterium, Pseudomonas sp.*
	TOMATO	*Pseudomonas fluorescens, Pseudomonas aeruginosa, Bacillus cereus, Bacillus megaterium, Bacillus circulans, Bacillus polymixa, Pseudomonas sp.,* Actinomycetes Series Albus
V2	CUCUMBER	*Pseudomonas fluorescens, Bacillus circulans, Pseudomonas lemonnieri, Pseudomonas sp., Bacillus megaterium, Bacillus sphaericus, Micrococcus sp.*
	BASIL	*Pseudomonas fluorescens, Pseudomonas lemonnieri, Arthrobacter citreus, Bacillus circulans, Pseudomonas sp., Bacillus subtilis,Bacillus megaterium, Bacillus sphaericus, Pseudomonas pseudogleyi*
	TOMATO	*Pseudomonas fluorescens, Pseudomonas sp., Bacillus circulans, Bacillus megaterium, Arthrobacter citreus, Micrococcus sp.*
V3	CUCUMBER	*Pseudomonas fluorescens, Bacillus megaterium, Arthrobacter globiformis, Arthrobacter simplex, Pseudomonas pseudogleyi, Micrococcus sp., Bacillus sphaericus*
	BASIL	*Pseudomonas fluorescens, Pseudomonas sp., Bacillus megaterium, Bacillus circulans, Bacillus sphaericus*
	TOMATO	*Bacillus megaterium, Bacillus cereus, Pseudomonas fluorescens, Pseudomonas sp., Micrococcus sp.*
V4	CUCUMBER	*Pseudomonas fluorescens, Pseudomonas pseudogleyi, Bacillus subtilis, Pseudomonas sp., Bacillus megaterium, Bacillus circulans, Bacillus cereus, Bacillus sphaericus, Arthrobacter globiformis, Bacillus polymixa, Arthrobacter simplex, Pseudomonas lemonnieri*
	BASIL	*Pseudomonas fluorescens, Pseudomonas sp., Bacillus circulans, Arthrobacter citreus, Bacillus polymixa, Pseudomonas pseudogleyi*
	TOMATO	*Pseudomonas fluorescens, Pseudomonas sp., Bacillus circulans, Bacillus mesentericus, Micrococcus sp., Bacillus circulans, Bacillus megaterium, Bacillus cereus, Pseudomonas lemonnieri, Bacillus subtilis*

Table 2. Taxonomic composition of fungal microflora

Experimental Variant		Fungal microflora Taxonomic composition
V1	CUCUMBER	*Actinomucor elegans, Trichoderma viride, Penicillium sp.*
	BASIL	*Trichoderma viride, Penicillium sp., Penicillium aurantiogriseum, Penicillium janthinellum*
	TOMATO	*Fusarium sp., Penicillium sp., Aspergillus ochraceus, Trichoderma harzianum*
V2	CUCUMBER	*Trichoderma viride, Penicillium sp., Penicillium janthinellum*
	BASIL	*Trichoderma viride, Trichoderma harzianum, Penicillium sp., Penicillium janthinellum*
	TOMATO	*Penicillium sp., Cladosporium herbarum, Monascus ruber*
V3	CUCUMBER	*Fusarium sp., Trichoderma harzianum, Penicillium sp.,Aspergillus versicolor, Cladosporium herbarum, Fusarium culmorum, Arthrobotrys oligospora*
	BASIL	*Trichoderma viride, Penicillium sp., Monascus ruber, Aspergillus versicolor, Paecilomyces sp., Aspergillus ochraceus*
	TOMATO	*Arthrobotrys arthrobotryoides, Fusarium sporotrichioides, Aspergillus versicolor, Acremonium strictum, Penicillium sp., Penicillium aurantiogriseum, Fusarium sp., Cladosporium herbarum*
V4	CUCUMBER	*Trichoderma viride, Trichoderma sp., Cladosporium herbarum, Penicillium sp.*
	BASIL	*Trichoderma harzianum, Penicillium sp., Acremonium strictum, Fusarium oxysporum, Aspergillus sp.*
	TOMATO	*Penicillium sp., Paecilomyces marquandii, Trichoderma harzianum*

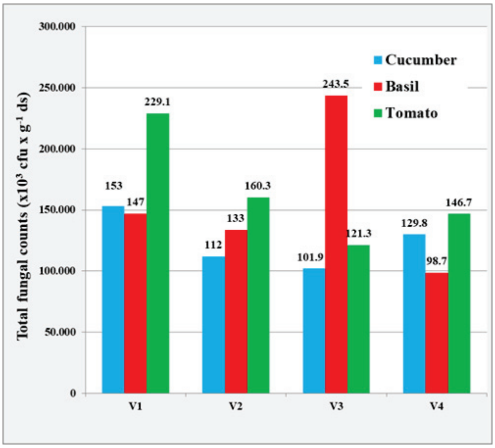

Figure 2. The effect of structured water on fungal counts

Analysis of data obtained in experiment show the beneficial effect of structured water on rhizosphere microflora of test plants by increasing bacterial counts and species diversity and decreasing fungal effectives below the values critical for plant health.

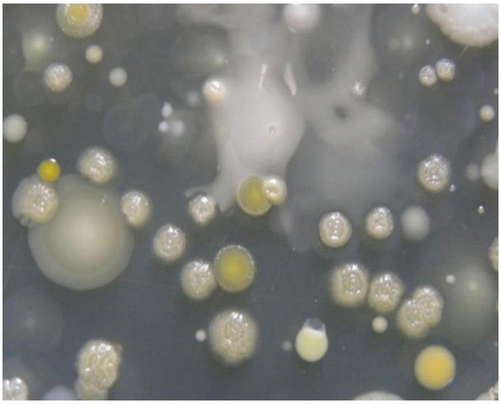

Figure 3. Bacterial microflora from experimental variant with structured water integral – Cucumber rhizosphere (Nutrient agar medium, 5 days)

Figure 4. *Trichoderma viride* from experimental variant with structured water integral best dilution – Cucumber rhizosphere (Czapek medium, 5 days)

Figure 5. The greenhouse experiment

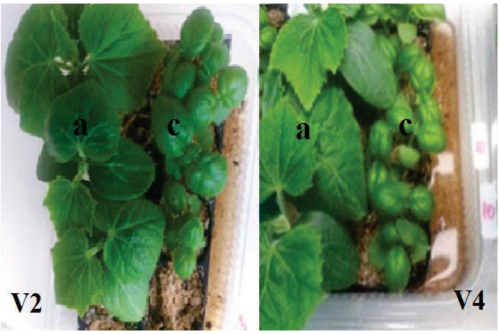

Figure 6. The effect of structured water V2 (left) and V4 (right) on biomass accumulated by test plants (a. cucumber and c. basil)

Development of antagonistic species of bacteria and fungi was noticed when structured water was administered as compared with control variant watered with tap water.

Consequent to re-equilibration of microbiota, a stimulating effect on accumulation of plant biomass was registered in variants V2 with structured water integral and V4 with structured water dilution no. 10, especially for cucumber and basil (Figures 5, 6 and Tables 3 and 4).

Table 3. Plant mass and root mass at cucumber (a) tomatoes (b) and basil (c) transplants

Variants	Plant mass (g)	Percent to control (%)	Root mass (g)	Percent to control (%)
V1 - a	4.10	100	1.03	100
V1 - b	3.38	100	1.59	100
V1 - c	1.30	100	0.53	100
V2 a	4.87	118.78	1.27	123.30
V2 b	3.97	117.46	1.85	116.35
V2 c	1.64	126.15	0.58	109.43
V3 –3 a	8.54	208.29	3.17	307.77
V3 –3 b	4.26	126.04	1.63	102.52
V3 –3 c	0.97	74.62	0.77	145.28
V4-10 a	4.63	112.93	0.63	61.17
V4-10 b	3.94	116.57	1.65	103.77
V4-10 c	1.24	95.38	0.47	88.68

Table 4. Foliar surfaces at cucumber (a) tomatoes (b) and basil (c) transplants

Variants	Foliar surfaces (cm^{2})	Percent to control (%)
V1 - a	57.53	100
V1 - b	6.70	100
V1 - c	6.50	100
V2 a	66.87	116.24
V2 b	12.80	191.04
V2 c	9.60	147.69
V3 –3 a	85.76	149.08
V3 –3 b	9.00	134.33
V3 –3 c	14.00	215.38
V4-10 a	74.82	130.06
V4-10 b	8.80	131.34
V4-10 c	14.30	220.00

The results in our experiment are in concordance with data from literature and field observation which report a beneficial effect of structured water on plants growth, health, yields quantity and quality (Dubey et al., 2018). Similarly, basil plants watered with structured water grew larger and proved to be more robust and resistant to summer heat in Arizona than plants watered with tap water (Abraham, 2014). In our experiment, the best results were obtained for watering with structured water from V4, where the foliar surface of basil plants was 220% to control, followed by V3 with 215% to control.

Other important effects on foliar surface were registered at V2 for tomato plants (191.04% to control) and V3 for cucumber (141.08 % to control). At this variant (V3), cucumber accumulated the highest plants mass (208.29% to control) and root mass (307.77% to control).

Private and commercial growers in Texas conducting plant experiments obtained improvements in the quantity, quality and health of products when watered with structured water (produced with NAT Structured Water Units from Natural Action Technologies, Inc.) for cucumber, tomato and strawberry as test plants.

They used 20-30% less water than usual and yielded an abundant crop of fruits with increased nutrient density (Brix measurements) and increased shelf life (Nolte, 2019).

CONCLUSIONS

It had significantly increased bacterial counts and species number registered for all test plant species under the influence of structured water and dilution 10 as compared to control.

The highest number of species (12) identified in rhizosphere of cucumber from structured water dilution 10 dominated by *Pseudomonas* (indicating improved qualitative conditions) and *Bacillaceae*.

High values of fungal counts from control and variant structured water no. 3 indicated less favourable conditions for communities where potential plant pathogen *Fusarium* developed.

Development of *Trichoderma* and *Paecilomyces* (antagonists for *Fusarium*) as well as other cellulolytic fungi active in rhizosphere were stimulated in variants with structured water integral and structured water dilution no. 10.

The experimental results confirmed the initial hypothesis and evidenced that structured water exhibited plant growth promoting properties, as a function of plant species involved and the variant (non-diluted or diluted).

Significantly higher root mass, foliar surface and total plant biomass accumulation were registered for treatments with structured water (structured water integral and diluted - variant 10) comparatively with control, especially for cucumber and basil.

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