Bulgarian Journal of Agricultural Science, 30 (Suppl. 1) 2024
Agricultural Academy

Wheat stalk growth promotion by a highly diluted fertilizer

Abraham O. Rodríguez-De la Fuente[1], Jose Antonio Heredia-Rojas[1], Ricardo Gomez-Flores[1], Michaela N. Beltcheva[2*], Diego Alan Carlos-Ramos[1], Pedro Antonio Noguera-Díaz López[1] and Omar Heredia-Rodríguez[1]

[1] *Autonomous University of Nuevo León, Faculty of Biological Sciences, Nuevo León, Mexico*
[2] *Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria*
[*] *Corresponding author:* mnedialkova@gmail.com

Abstract

Rodríguez-De la Fuente, A. O., Heredia-Rojas, J. A., Gomez-Flores, R., Beltcheva, M. N., Carlos-Ramos, D. A., Noguera-Díaz López, P. A. & Heredia-Rodríguez, O. (2024). Wheat stalk growth promotion by a highly diluted fertilizer. *Bulg. J. Agric. Sci., 30 (Supplement 1)*, 59–62

Alternatives to using fertilizers in modern agriculture have prompted research of new approaches such as ultra-diluted solutions. The present study aimed to evaluate the effect of homeopathic plant fertilizer preparations, made via traditional stepwise dilution and succussion procedures, on wheat stalk growth. We used 10^{-7}, 10^{-14}, and 10^{-36} dilutions of a commercial fertilizer and *Triticum durum* Rafi C97 variety wheat grains, which were compared with the negative controls (*a*) pure bi-distilled water and (*b*) sham-treated pure bi-distilled water, following the stepwise dilution and succussion method, and a 2% commercial fertilizer solution as a positive control. Each treatment consisted of 120 grains, distributed across 10 disposable Petri dishes. Stalk length was measured by an electronic Vernier device and reported as total length in centimeters. Three independent experiments were conducted, each including all dilutions and controls. Statistical differences were determined by analysis of variance and using the Tukey test to establish individual differences. Results showed a significant ($p < 0.05$) increased on wheat stalk growth after treatment with diluted fertilizer solutions, as compared with negative controls. Positive control showed the highest stalk growth, but it was not statistically different from that of diluted fertilizer treatments. Our findings suggest that the highly diluted commercial fertilizer promoted wheat seedlings growth, highlighting potential benefits for sustainable agricultural practices. However, additional experiments analyzing this effect at different phases of plant development are necessary, and more endpoints need to be considered.

Keywords: homeopathy; fertilizers; sustainable agriculture; wheat growth

Introduction

Drugs at ultra-high dilutions, close to Avogadro's number (theoretical 0-molarity is 10^{-24}), and without apparently containing any drug molecule, have been used in homeopathy therapy for about two centuries. Nowadays, homeopathic procedures involving the controversial phenomenon called "water memory" have regained their use in different application areas. Basic research on homeopathy involves several aspects. However, investigations have been mainly focused on the potentization or dynamization principle (Mathie, 2019), and evidence has been mounting for substance homeopathic ultra-dilutions specific effects (Endler et al., 2010; Betti et al., 2013; Munshi et al., 2019). In this regard, medical applications and clinical studies on therapeutic and prophylactic effects have been demonstrated (Sukul and Sukul, 2006; Bracho et al., 2010). On the other hand, increasing agricultural use of homeopathic preparations for plant growth stimulation has been reported (Brizzi et al., 2005; Pfleger et al., 2011; Pulido et al., 2017; Ücker et al., 2018; Abasolo-Pacheco et al., 2020).

In the present study, 10^{-7}, 10^{-14}, and 10^{-36} dilutions of a commercial fertilizer were evaluated in a 7-d wheat growth bioassay. Given this relevant issue involving the biological effects of homeopathic preparations, mainly based on the stepwise dilution and agitated succussion method, as shown by the German physician Samuel Hahnemann (Schmidt, 2010), who introduced homeopathy as a therapeutic system, the present study aimed to evaluate a highly diluted commercial fertilizer on an *in vitro* wheat stalk growth model.

Material and Methods

Homeopathic preparations

We prepared a 2% stock solution of the commercial fertilizer Vita-Plant Nutrition (Heroes de Padierna, Ciudad de México, Estado de México, México), containing a complex mixture of nutrients and salts. One milliliter of this solution was diluted in 9 mL of pure bi-distilled water in a glass bottle and 7, 14, and 36 steps of agitated dilutions against an elastic surface (200 strokes of vigorous turbulent shaking were made for each step of dilution) were performed to obtain 10^{-7}, 10^{-14}, and 10^{-36} dilutions.

Bioassays

We used a previously reported 7-d wheat growth bioassay (Pfleger et al., 2011) for evaluating the homeopathic dilutions. Herbicide-and pesticide-free *Triticum durum* Rafi C97 variety wheat grains were purchased at a local supplier and allocated in disposable Petri dishes; 120 grains were equally divided into 12 plates, containing two layers of 90 mm Whatman grade 2 cellulose filter paper (Piscataway, NJ, USA). Plates were then covered and placed in alternating rows according to a random model (stratified randomization) in a bio-climatic chamber at 27 °C ± 0.3 °C and 45% of relative humidity, with a photoperiod of 12 h darkness/12 h light. Wheat grains were not soaked before treatments. During the bioassay, grains were watered every 24 h by adding pure bi-distilled water or treatments. Stalks were cut off to individual measurements after seven days of treatment. Total stalk length was blindly evaluated by an electronic Vernier device and expressed as total length (cm). Experimental groups were harvested in the same sequence as they were planted.

Experimental design

We used diluted and agitated preparations (10^{-7}, 10^{-14}, and 10^{-36} dilutions) of the stock solution as explained above, using the negative controls pure bi-distilled water and sham-treated pure bi-distilled water (prepared under the same procedure used for the fertilizer dilutions). In addition, 2% of a commercial fertilizer (Vita-Plant Nutrition) was used as a positive control. Three independent experiments were performed, providing 360 analyzed grains per treatment and controls. Each independent experiment included the same set of dilutions and controls, which were encoded and blindly applied, and evaluated. At the end of the experiment, codes were revealed, and data statistical analysis was performed.

Statistical analysis

Statistical differences among groups were determined by analysis of variance for normal distributions and the correspondent Tukey test for establishing individual differences. Data normality was calculated by the Kolmogorov-Smirnov test ($p < 0.05$). Results were expressed as arithmetical means ± SD of all 120 measurements and processed with the SPSS v.15.0 package (Chicago, IL, USA).

Results and Discussion

In the present study, we determined the effect of 10^{-7}, 10^{-14}, and 10^{-36} dilutions of a commercial fertilizer on an *in vitro* model of stalk wheat growth. As is shown in Figure 1, the highly diluted fertilizer and positive control (2% commercial fertilizer) groups presented significantly ($p < 0.05$) higher wheat stalk lengths, as compared with the negative and sham-treated controls. Although positive control produced the highest ($p < 0.05$) wheat stalk growth, it was no different from that of highly diluted and agitated treatments.

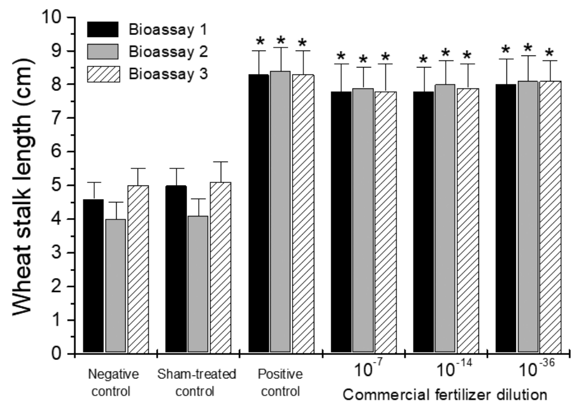

Fig. 1. Effect of highly diluted-agitated commercial fertilizer on wheat stalk length (cm)

We used 120 wheat grains for the treatments and controls. The negative control was the vehicle (untreated pure bi-distilled water), whereas the sham-treated control included pure bi-distilled water processed under the same protocol for fertilizer dilutions, as explained in the text. We used 2% of the original commercial fertilizer as a positive control. Bars represent arithmetical means ± SD of triplicate determinations from three independent bioassays. *$p < 0.05$ as compared with the negative and sham-treated controls

There is a trend towards using homeopathic procedures in various areas of science and applications in diverse fields. Considering this trend and the lack of consensus on the effectiveness of the techniques involving ultra-diluted chemical compounds, it is of considerable interest to examine the potential biological effects of ultra-diluted solutions. In the present study, homeopathic preparations made according to the traditional method of stepwise dilution and succussion, as derived from traditional homeopathy, were evaluated by using a previously described and validated wheat growth bioassay (Pfleger et al., 2011).

It has been proposed that the phenomenon known as "water memory" contributes to the high dilution process (Thomas, 2007). However, this issue is controversial, and some researchers claim that any interpretation calling for memory effects in pure water should be excluded (Teixeira, 2007), or at least is an elusive phenomenon (Colic and Morse, 1999). Regarding this controversial phenomenon of water memory, we have previously demonstrated that water mimics the behavior of a chemical substance by electronic transmission of the original substance to water samples, via an electronic amplifier (Heredia-Rojas et al., 2011, 2012, 2015). We tested the biological effect of pure water samples treated in a bioresonance instrument to inhibit the growth of *Entamoeba histolytica* and *Trichomonas vaginalis* (Heredia-Rojas et al., 2011), fungus of clinical importance (Heredia-Rojas et al., 2012), and bacteria (Heredia-Rojas et al., 2015) by electrotransferring metronidazole, fungizone, and vancomycin to water, respectively. Our research group has hypothesized that water possesses memory based on the biological activity of such water samples transferred with electronic information from the original compound (Norman et al., 2016). Moreover, this controversial phenomenon of water memory has been gaining scientific credibility, either through direct empirical findings such as results presented in this work or by theoretical considerations (Stock-Schröer et al., 2009).

In compliance with the Avogadro number, the potency of 10^{-36} used in the present study contains only the molecules of the diluent medium, in this case, water. As observed in all Figures, we assumed that wheat stalk growth was stimulated by the influence of diluted fertilizer, as compared with negative control and with sham-treated water samples. These results agreed with previous reports indicating the biological activity of drugs at high dilutions, even those that exceeded Avogadro's limit (Boujedaini et al., 2012; Şeker et al., 2018).

Regarding the mode of action or behavior of the homeopathic potencies, attempts have been made to explain the physical basis of this phenomenon. Sukul & Sukul (2006), using nuclear magnetic resonance spectroscopy indicated that potentized drugs differ from each other and the medium (vehicle) concerning the spin-lattice relaxation time; infrared spectra of potentized drugs showed variation in the vibrational frequencies of O-H, C-O, and C-H bands. Furthermore, Fourier transform infrared of potentized drugs demonstrated marked variation in O-H bending vibration. Moreover, electronic and fluorescence spectra of homeopathic preparations presented variations in spectral patterns, peaks, and absorbance or intensities. These findings indicate the variation in hydrogen bonding and H-bonding strength among the potencies (Sukul and Sukul, 2006). By using thermoluminescence techniques, Rey (2002) reported that lithium chloride and sodium chloride ultra-high dilutions showed changes among them. On the other hand, Demangeat (2015) suggested that gas nanobubbles and aqueous nanostructures play a critical role in the dynamization process. Taken together, we selected to follow the traditional diluted and agitated procedure to prepare homeopathic dilutions. Thus, by applying 200 strokes of vigorous turbulent shaking to dilutions, it was possible to create enough of such nanobubbles by mechanical action. On the other hand, it is well known that increasing organic matter content in soil due to the application of chemical fertilizers is of ecotoxicological concern because potentially toxic elements accumulate and become available for crops (Iglesias et al., 2018). Thus, it is relevant to develop approaches, such as the one presented in this study, to avoid the use of large amounts of chemicals in soils.

We did not dilute a specific substance, but a complex mix of several elements that according to the fertilizer manufacturer, function as a growth stimulator for crops and other plants. In contrast, most studies testing high-diluted substances use specific and isolated chemical compounds (Teixeria &Carneiro, 2017).

Conclusions

The current study demonstrated that 10^{-7}, 10^{-14}, and 10^{-36} dilutions of a commercial fertilizer significantly stimulated *in vitro* wheat stalk growth. These findings indicated that "fertilizer information" remained in the diluted sample, even at 10^{-36}, which is beyond Avogadro´s number. These findings suggest that the highly diluted commercial fertilizer influenced the growth of wheat seedlings, highlighting potential benefits for sustainable agricultural practices. However, further studies analyzing this effect at distinct phases of plant development, studies at larger scales, and more endpoints need to be considered to confirm our results.

Acknowledgements

We would like to thank Dr. Pedro Freeze Flores for his directions in preparing the homeopathic dilutions.

Conflict of interest

The authors have declared that no competing interests exist.

References

Abasolo-Pacheco, F., Ojeda-Silvera, C. M., García-Gallirgos, V., Melgar-Valdes, C., Nuñez-Cerezo, K. & Mazón-Suástegui, J. M. (2020). Effect of homeopathic medicines during the initial stage and vegetative development of cucumber plants (*Cucumis sativus* L.). *Terra Latinoamericana, 38*(1), 165-180.

Betti, L., Trebbi, G., Olioso, D., Marzotto, M. & Bellavite, P. (2013). Basic Research in Homeopathy and Ultra-High Dilutions: What Progress is Being Made? *Homeopathy, 102*,151-154.

Boujedaini, N., Terzan, L. & Khuda-Bukhsh, A. R. (2012). Summary of Four Scientific studies on Arsenicum Album High Dilution Effect Against Arsenic Intoxication in Mice. *International Journal of High Dilution Research, 11*, 150-151.

Bracho, G., Varela, E., Fernández, R., Ordaz, B., Marzoa, N., Menéndez, J., García, L., Gilling, E., Leyva, R., Rufín, R., De La Torre, R., Solis, R. L., Batista, N., Norrero, R. & Campa, C. (2010). Large-Scale Application of Highly-Diluted Bacteria for Leptospirosis Epidemic Control. *Homeopathy, 99*(3), 156-166.

Brizzi, M., Lazzarato, L., Nani, D., Borghini, F., Peruzzi, M. & Betti, L. (2005). A Biostatistical Insight into the As2O3 High Dilution Effects on the Rate and Variability of Wheat Seedling Growth. *Complementary Medicine Research, 12*(5), 277-283.

Colic, M. & Morse, D. (1999). The Elusive Mechanism of the Magnetic "Memory" of Water. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 154*,167-174.

Demangeat, J. (2015). Gas Nanobubbles and Aqueous Nanostructures: The Crucial Role of Dynamization. *Homeopathy, 104*,101-115.

Endler, P. C., Thieves, K., Reich, C., Matthiessen, P., Bonamin, L., Scherr, C., Baumgartner, S. (2010). Repetitions of Fundamental Research Models for Homeopathically Prepared Dilutions Beyond 10-23: A Bibliometric Study. *Homeopathy, 99*(1), 25-36.

Heredia-Rojas, J. A., Torres-Flores, A. C., Rodríguez-De La Fuente, A. O., Mata-Cárdenas, B. D., Rodríguez-Flores, L. E., Barrón-González, P., Torres-Pantoja, A. C. & Alcocer-González, J. M. (2011). *Entamoeba histolytica* and *Trichomonas vaginalis*: Trophozoite Growth Inhibition by Metronidazole Electrotransferred Water. *Experimental Parasitolology, 127*, 80-83.

Heredia-Rojas, J. A., Gomez-Flores, R., Rodríguez-De La Fuente, A. O., Monreal-Cuevas, E., Cayetano Torres-Flores, A., Rodriguez-Flores, L. E. & Beltcheva, M. (2012). Antimicrobial Effect of Amphotericin B Electronically-Activated Water Against *Candida Albicans*. *African Journal of Microbiology Research, 6*(15), 3684-3689.

Heredia-Rojas, J. A., Villarreal-Treviño, L., Rodríguez-De La Fuente, A. O., Herrera-Menchaca, L. I., Gomez-Flores, R., Mata-Cárdenas, B. D. & Rodríguez-Flores, L. E. (2015). Antimicrobial Effect of Vancomycin Electro-Transferred Water Against Methicillin-Resistant *Staphylococcus aureus* Variant. *African Journal of Traditional, Complementary and Alternative Medicines, 12*(1), 104-108.

Iglesias, M., Marguí, E., Camps, F. & Hidalgo, M. (2018). Extractability and Crop Transfer of Potentially Toxic Elements from Mediterranean Agricultural Soils Following Long-Term Sewage Sludge Applications as a Fertilizer Replacement to Barley and Maize Crops. *Waste Management, 75*, 312-318.

Mathie, R. T. (2019). Diversity and Excellence In Homeopathy Research. *Homeopathy, 108*(2), 75.

Munshi, R., Joshi, S., Talele, G. & Shah, R. (2019). An In-Vitro Assay Estimating Changes in Melanin Content of Melanoma Cells Due to Ultra-Dilute, Potentized Preparations. *Homeopathy, 108*(3), 183-187.

Norman, R. L., Dunning-Davies, J., Heredia-Rojas, J. A. & Foletti, A. (2016). Quantum Information Medicine: Bit as it—The Future Direction of Medical Science: Antimicrobial and Other Potential Nontoxic Treatments. *World Journal of Neuroscience, 6*(3), 193-207.

Pfleger, A., Hofäcker, J., Scherer-Pongratz, W., Lothaller, H., Reich, C. & Endler, P. C. (2011). The Effect of Extremely Diluted Agitated Gibberellic Acid (10e−30) on Wheat Stalk Growth. A Two Researcher Pilot Study. *Complementary Therapies in Medicine, 19*(3), 164-169.

Pulido, E., Boff, P., Duarte, T. & Boff, M. I. (2017). High dilution preparations for organic production system of broccoli. *Agron. Colomb., 35*, 53-58.

Rey, L. (2002). Thermoluminescence of Ultra-High Dilutions of Lithium Chloride and Sodium Chloride. *Physica A*, *323*, 67-74.

Schmidt, J. M. (2010). 200 years Organon of Medicine–A comparative Review of its six editions (1810–1842). *Homeopathy, 99*(4), 271-277.

Şeker, S., Güven, C., Akçakaya, H., Bahtiyar, N., Akbaş, F. & Onaran, I. (2018). Evidence that Extreme Dilutions of Paclitaxel and Docetaxel Alter Gene Expression Of In Vitro Breast Cancer Cells. *Homeopathy, 107*(1), 32-39.

Stock-Schröer, B., Albrecht, H., Betti, L., Endler, P.C., Linde, K., Lüdtke, R., Musial, F., Van Wijk, R., Witt, C. & Baumgartner, S. (2009). Reporting Experiments in Homeopathic Basic Research (Rehbar) – A Detailed Guideline for Authors. *Homeopathy, 98*(4), 287-298.

Sukul, N. C. & Sukul, A. (2006). Preparation of High Dilutions of Drugs. In High Dilution Effects: Physical and Biochemical Basis, Kluwer Academic Publishers, Dordrecht, 3-10.

Teixeira, J. (2007). Can Water Possibly Have a Memory? A Sceptical View. *Homeopathy, 96*(3), 158-162.

Teixeria, M. & Carneiro, S. (2017). Effects of Homeopathic High Dilutions on Plants: Literature Review. *Revista de Homeopatía, 80*(3-4), 104-120.

Thomas, Y. (2007). The History of the Memory of Water. *Homeopathy, 96*(3), 151-157.

Ücker, A., Baumgartner, S., Sokol, A., Huber, R., Doesburg, P. & Jäger, T. (2018). Systematic review of plant-based homeopathic basic research: an update. *Homeopathy, 107*(2), 115-129.

Received: **October, 09, 2024**; *Approved*: **November, 07, 2024;** *Published:* **December, 2024**