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In Vitro Increased Wheat Stalk Growth by Highly Diluted Agitated Preparations of a Commercial Fertilizer

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Abstracts. Drugs at ultra-high dilutions close to the 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. 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. Test dilutions were prepared following a standardized protocol, according to the method of stepwise dilution and succession, as derived from traditional homeopathy. Experiments were performed on herbicides- and pesticides-free wheat grains (*Triticum durum* Rafi C97 variety). Treatments were compared with negative controls including; a) pure bi-distilled water and b) sham-treated pure bi-distilled water, following the same protocol as used for fertilizer dilutions with 200 strokes and the same steps of dilutions. A 2% of commercial fertilizer was used as a positive control. The observed results of three independent bioassays, showed an increased wheat stalk growth after treatment with diluted and agitated fertilizer solutions, as compared with negative controls ($p < 0.05$). Positive control showed the highest stalk growth, but it was not significantly different from that of diluted fertilizer. These results suggest that there was an influence of highly diluted commercial fertilizer on wheat seedling development.

Key words: Wheat growth, Sustainable agriculture, Fertilizers.

Introduction

Samuel Hahnemann (1755-1843) introduced homeopathy as a therapeutic system in his book "*Organon of the rationale System of Medicine*". Nowadays, 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; Pfleger et al., 2011; Betti, et al., 2013; Munshi et al., 2019). In this regard, medical applications and clinical studies on therapeutic and prophylactic effects have been reported (Sukul & Sukul, 2006; Bracho et al., 2010). On the other hand, agricultural use of homeopathy for plant growth and germination stimulation (Brizzi et al., 2005) and against plant pests (Betti et al., 2013; Wyss et al., 2010) has significantly increased.

In view of this relevant issue involving biological effects of homeopathic preparations, mainly based in the stepwise dilution and agitated succussion method, as shown by Hahnemann, the present study aimed to evaluate a highly diluted commercial fertilizer on *in vitro* wheat stalk growth.

Materials and Methods

Homeopathic preparations

Generally, water and ethanol are proper ingredients to generate homeopathic potentized drugs. However, it is well recognized that water does not retain the efficacy of a potentized drug (Boyd, 1954), for which, in this study, highly diluted and agitated preparations were immediately used after succussions. To prepare test dilutions, a 2% stock solution of the liquid commercial fertilizer Vita-Plant Nutrition®, purchased from a local supplier and containing a complex mixture of nutrients and salts, was prepared and considered as "mother substance"; this concentration was selected based in our previous bioassays (data not shown). Next, 1 mL of the mother substance was added to 9 mL of bi-distilled water in a glass bottle. The dilution was vigorously agitated against an elastic surface (200 strokes of vigorous turbulent shaking were made for each step of dilution) and 7, 14, and 36 steps of agitated dilutions were performed to obtain 10^{-7}, 10^{-14}, and 10^{-36} dilution treatments.

Bioassays

As previously described (Pfleger et al., 2011), a 7-d wheat growth bioassay was used to evaluate the biological effect of homeopathic dilutions. Herbicide- and pesticide-free wheat grains (*Triticum durum*; Rafi C97 variety harvested in 2018) were purchased at a local supplier and allocated in disposable Petri dishes; 120 grains were equally divided in 12 plates, containing 2 layers of filter paper (Whatman, cellulose, 90 mm, sort 2). Plates were then covered and placed in alternating rows according to a random model (stratified randomization) in a bio-climatic chamber at 27 ± 0.3 °C and 45% of relative humidity, with a photoperiod of 12 h darkness/12 h light. Wheat grains had not been soaked prior to treatment. 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 7 days of treatment. The total stalk length was evaluated by using an electronic Vernier instrument and expressed as total length (cm). Experimental groups were harvested in the same sequence as they were planted. Measurements were performed blindly. In this study, we followed recommendations provided by Stock-Schröer et al. (2009) in order to present objective information and avoid speculations.

Experimental design

We used diluted (10^{-7}, 10^{-14}, and 10^{-36}) and agitated conditions of the original substance, as explained above. The following controls were matched to every experiment: negative controls consisting of a) pure bi-distilled water and b) sham-treated pure bi-distilled water, following the same protocol as used for fertilizer dilutions with 200 strokes and the same steps of dilutions, and 2% of commercial fertilizer as a positive control. Three independent experiments, in a period of 18 months, were performed and every experiment included the same set of dilutions and controls, analyzing 360 grains for every treatment and controls, which were encoded and blindly applied and evaluated. At the end of the experiment, codes were revealed and data statistical analysis 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 ($p<0.05$). When no-normal distributions were found, a Kruskal-Wallis test was applied and the correspondent non-parametric Tukey test, using the SPSS v.15.0 package. Results were expressed as mean ± SD of triplicate determinations.

Results

In the present study, the effect of 10^{-7}, 10^{-14}, and 10^{-36} dilutions of a commercial fertilizer on an *in vitro* model of stalk wheat growth was evaluated. As shown in Figures 1 to 3, high dilution and positive control (2% commercial fertilizer) groups presented higher wheat stalk lengths, as compared with negative and sham-treated controls ($p<0.05$). Although positive control induced the highest ($p<0.05$) wheat stalk growth, it was no different from that of highly diluted agitated treatments.

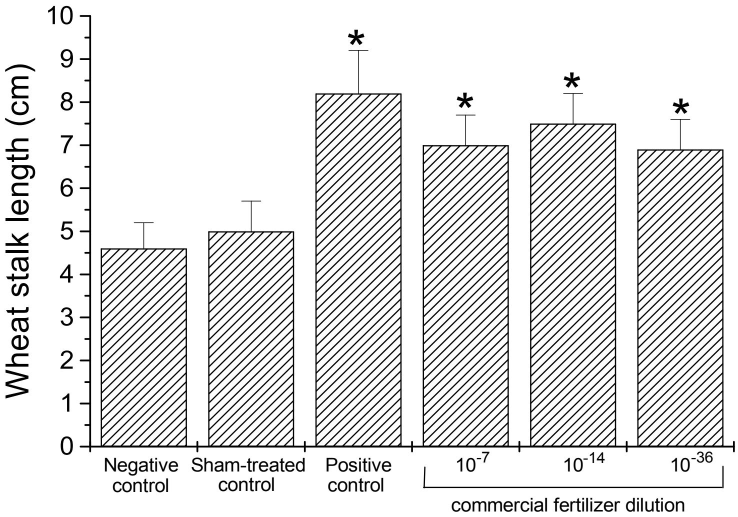

Fig. 1. Mean wheat stalk length (cm) under the influence of highly diluted-agitated fertilizer. One hundred and twenty wheat grains were used for treatments and controls. **Bioassay 1.** The negative control included only the vehicle (untreated pure bi-distilled water). Sham-treated control was processed following the same protocol as used for fertilizer dilutions, as explained in the text. A 2% of the original commercial fertilizer was used as a positive control. Bars represent mean ± SD of triplicate determinations. *$p < 0.05$ as compared with negative and sham-treated controls.

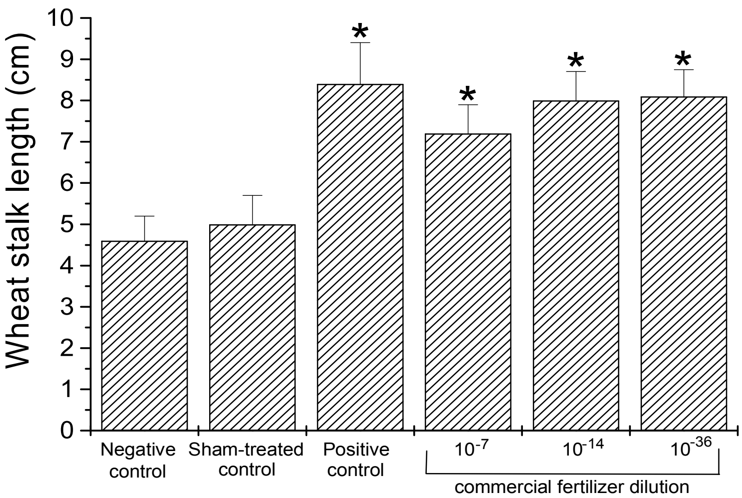

Fig. 2. Mean wheat stalk length (cm) under the influence of highly diluted-agitated fertilizer. One hundred and twenty wheat grains were used for treatments and controls. **Bioassay 2**. The negative control included only the vehicle (untreated pure bi-distilled water). Sham-treated control was processed following the same protocol as used for fertilizer dilutions, as explained in the text. A 2% of the original commercial fertilizer was used as a positive control. Bars represent mean ± SD of triplicate determinations. *$p < 0.05$ as compared with negative and sham-treated controls.

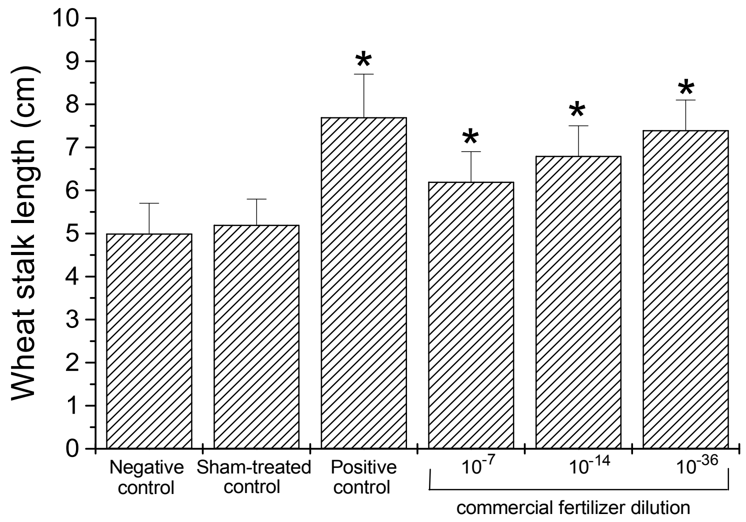

Fig. 3. Mean wheat stalk length (cm) under the influence of highly diluted-agitated fertilizer. One hundred and twenty wheat grains were used for treatments and controls. **Bioassay 2**. The negative control included only the vehicle (untreated pure bi-distilled water). Sham-treated control was processed following the same protocol as used for fertilizer dilutions, as explained in the text. A 2% of the original commercial fertilizer was used as a positive control. Bars represent mean ± SD of triplicate determinations. *$p < 0.05$ as compared with negative and sham-treated controls.

Discussion

There is a trend towards the use of homeopathic procedures in several areas of science and applications in a diversity of 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 tested 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” is involved in the high dilution process (Thomas, 2007). However, this issue is controversial and some investigations claim that any interpretation calling for memory effects in pure water is totally excluded (Teixeira, 2007) or at least is an elusive phenomenon (Colic & 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. 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 electro-transferring metronidazole, fungizone and vancomycin to water, respectively. We have hypothesized that water possesses memory based on 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 (Ruzic et al., 2008).

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 stalks growth was stimulated by influence of diluted fertilizer, as compared with negative control and sham-treated water samples. These results agreed with previous reports indicating biological activity of drugs at high dilutions, even those that exceeded the Avogadro's limit (Boujedaini et al., 2012; Seker et al., 2018).

Regarding the mode of action or behavior of the homeopathic potencies, attempts have been made in order 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 from the medium (vehicle) with respect to 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 variation with regard to spectral pattern, peaks and absorbance or intensities. All of these findings are indicative of the variation in hydrogen bonding and H-bonding strength among the potencies (Sukul & Sukul, 2006). By using thermoluminescence techniques, Rey (2002) reported that lithium chloride and sodium chloride ultra-high dilutions showed changes among them. By the way, Demangeat (2015), suggested that gas nanobubbles and aqueous nanostructures play a critical role in the dynamization process. Taken together, in the present study 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 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 quantities of chemicals in soils.

We did not dilute a specific substance, but a complex mix of several elements that according to the fertilizer manufacturer, act 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).

In conclusion, we 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 the Avogadro´s number. The potential use of high-diluted fertilizers for agricultural purposes is discussed. However, further studies at larger scales are required to confirm these results.

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