



• In vitro increased wheat stalk growth by highly diluted agitated preparations of a commercial fertilizer



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INTRODUCTION

Drugs at ultra-high dilution which cross the Avogadro number and apparently do not contain any drug molecule, have been used in the therapeutic system known as homeopathy for about a couple of centuries. Nowadays, the homeopathic procedures that involve the controversial phenomenon of the so-called “water memory”, have regained their use in different areas of application. Evidence has been shown for substance specific effects of homeopathic ultra-dilutions (Endler et al., 2010; Pfleger et al., 2011; Munshi et al., 2019). Agricultural applications of homeopathy have been raised in the recent years, for controlling plant pests (Betti et al., 2003; Wyss et al., 2010), and for plant growth and germination stimulation (Brizzi et al., 2000; Brizzi et al., 2005).

In view of this interesting issue involving biological effects of homeopathic preparations, mainly based in the method of stepwise dilution and agitated succussion, as derived from the traditional homeopathy described by Hahnemann, we aimed the current study to evaluate three high dilutions of a commercial trade-mark® fertilizer (10e-7, 10e-14 and 10e-36) by using of a wheat growth bioassay.

MATERIALS AND METHODS

Homeopathic preparations

For preparations of test dilutions, a 2% stock solution of a liquid commercial trademark® fertilizer (Vita-Plant Nutrition, purchased in a local supplier) was prepared and considered as “mother substance”, 1x. Then, using a disposable pipette, 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 (a total of 200 strokes of vigorous turbulent shaking were made for each step of dilution). A total of 7, 14, and 36 steps of agitated dilutions were carry out in order to have three different diluted treatments (7x, 14x, and 36x) of the original substance.

Bioassays

As previously described (Pfleger et al., 2011), a wheat growth bioassay 7-days was used to evaluate the biological effect of homeopathic dilutions. Stalk length was evaluated after 7 days. Stalks were cut off before to individual measurements. The total stalk length was evaluated by using an electronic Vernier instrument and expressed as total length (cm).

Experimental design

Three diluted and agitated conditions of the original substance (7x, 14x, and 36 x) were considered as explained above. Two controls were matched to each experiment: A negative control, including only the vehicle of pure bi-distilled water. Moreover, a sham-treated pure bi-distilled water was included following the same protocol as used for fertilizer dilutions with 200 strokes and the same steps of dilution. The original 2% of stock fertilizer solution was used as a positive control. Differences among groups were calculated by Analysis of Variance for normal distributions.

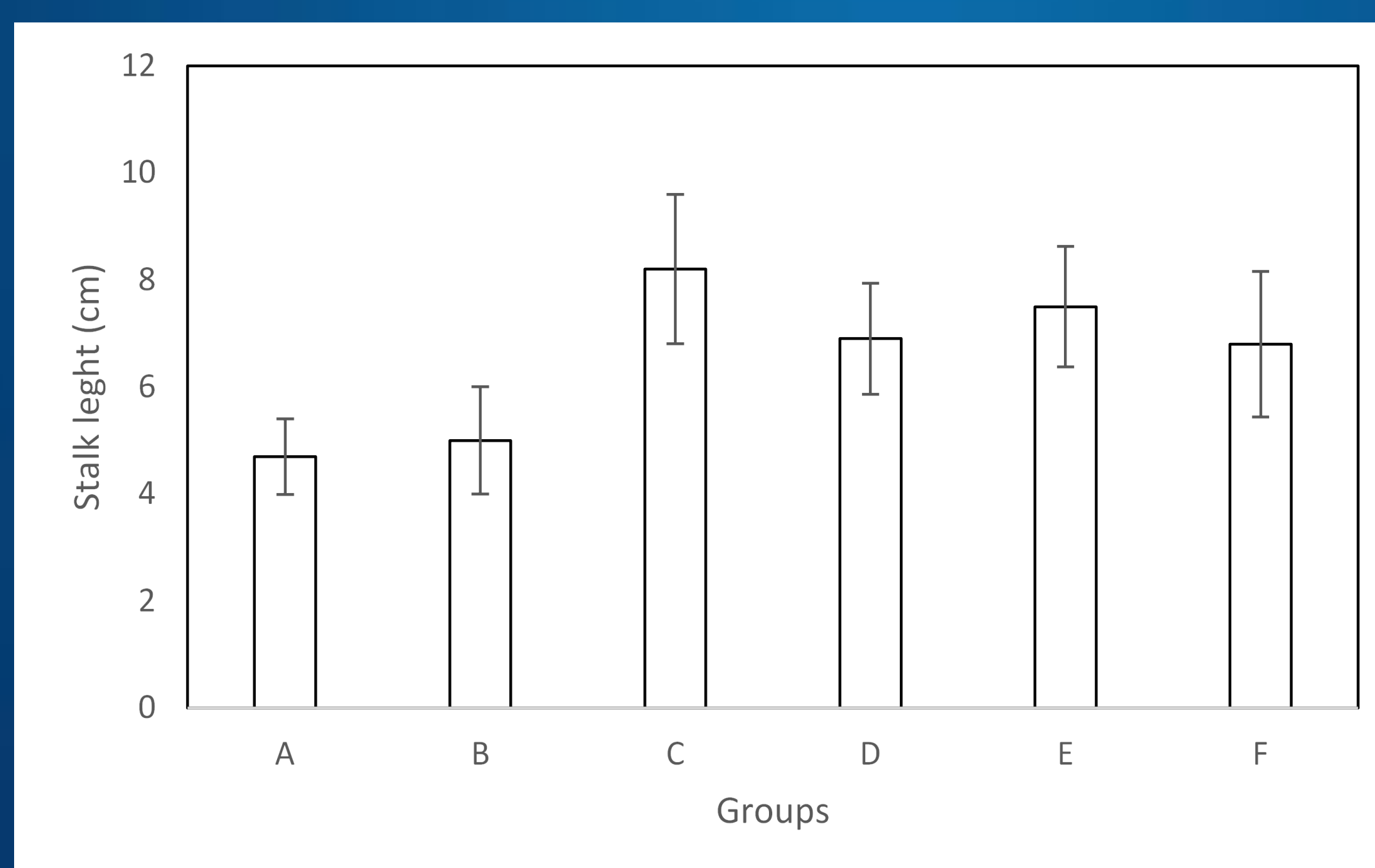


A general view of the wheat growth bioassay 7-days . Stalk total length was evaluated after 7 days under high-dilution fertilizer treatments.

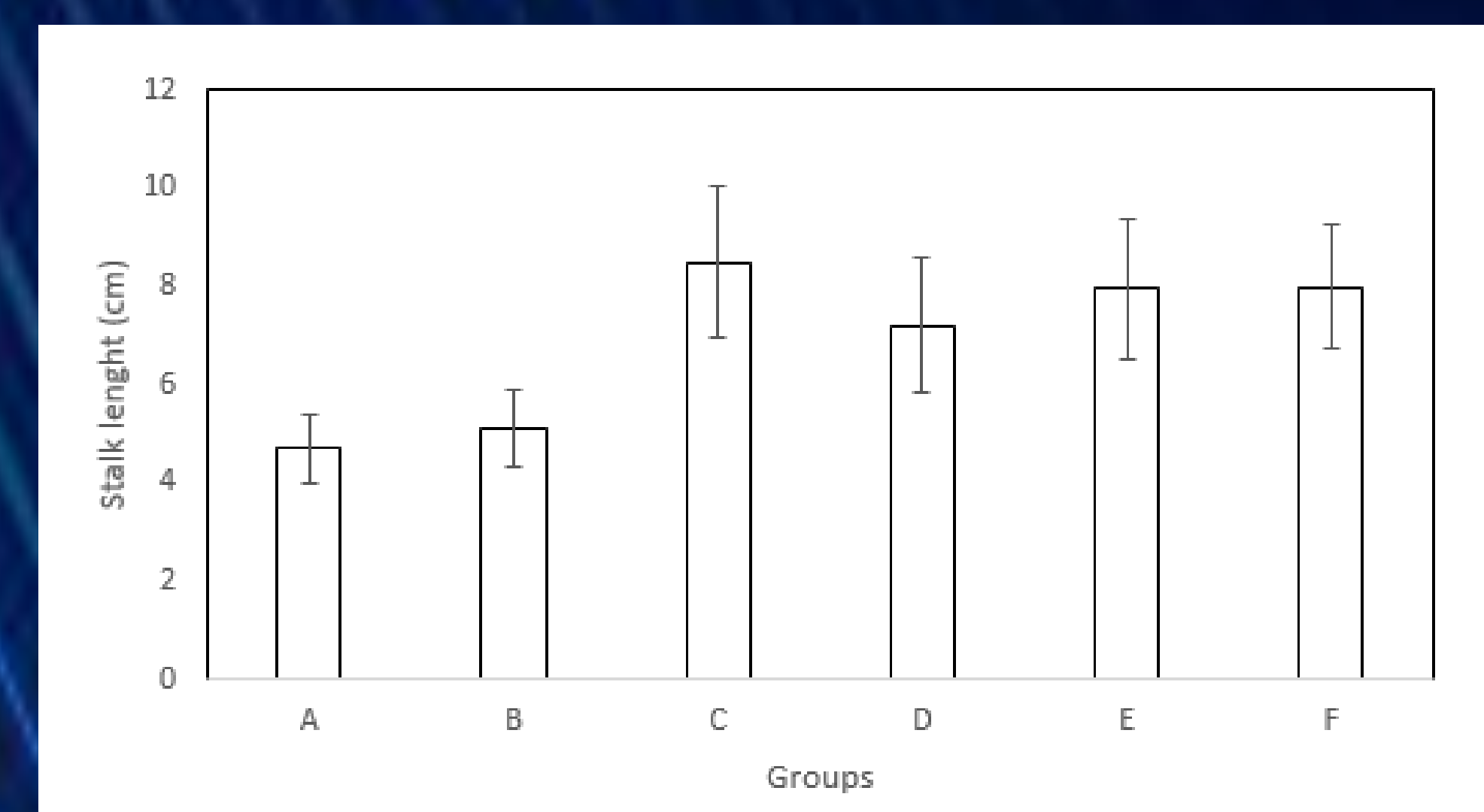
RESULTS AND DISCUSSION

The design of the present study evaluated the effect of three high diluted (10e-7, 10e-14 and 10e-36) water samples of a commercial fertilizer on a model of stalk wheat growth. The figure 1, illustrates the grouped means of stalk lengths for the treatment regimens and controls. In all cases, the high dilution groups presented higher stalk lengths when compared with negative and sham-treated water ($p < 0.05$). The positive control of 2% of the original fertilizer (stock solution), showed the highest values of stalk growth, as expected ($p < 0.05$). By the way, figures 2 and 3 shows the results from other two independent bioassays that were performed at different time, indicating the same effect in stimulating stalk growth compared with controls ($p < 0.05$) as the same results found for experiment 1. The three independent bioassays were performed in a period of 18 months.

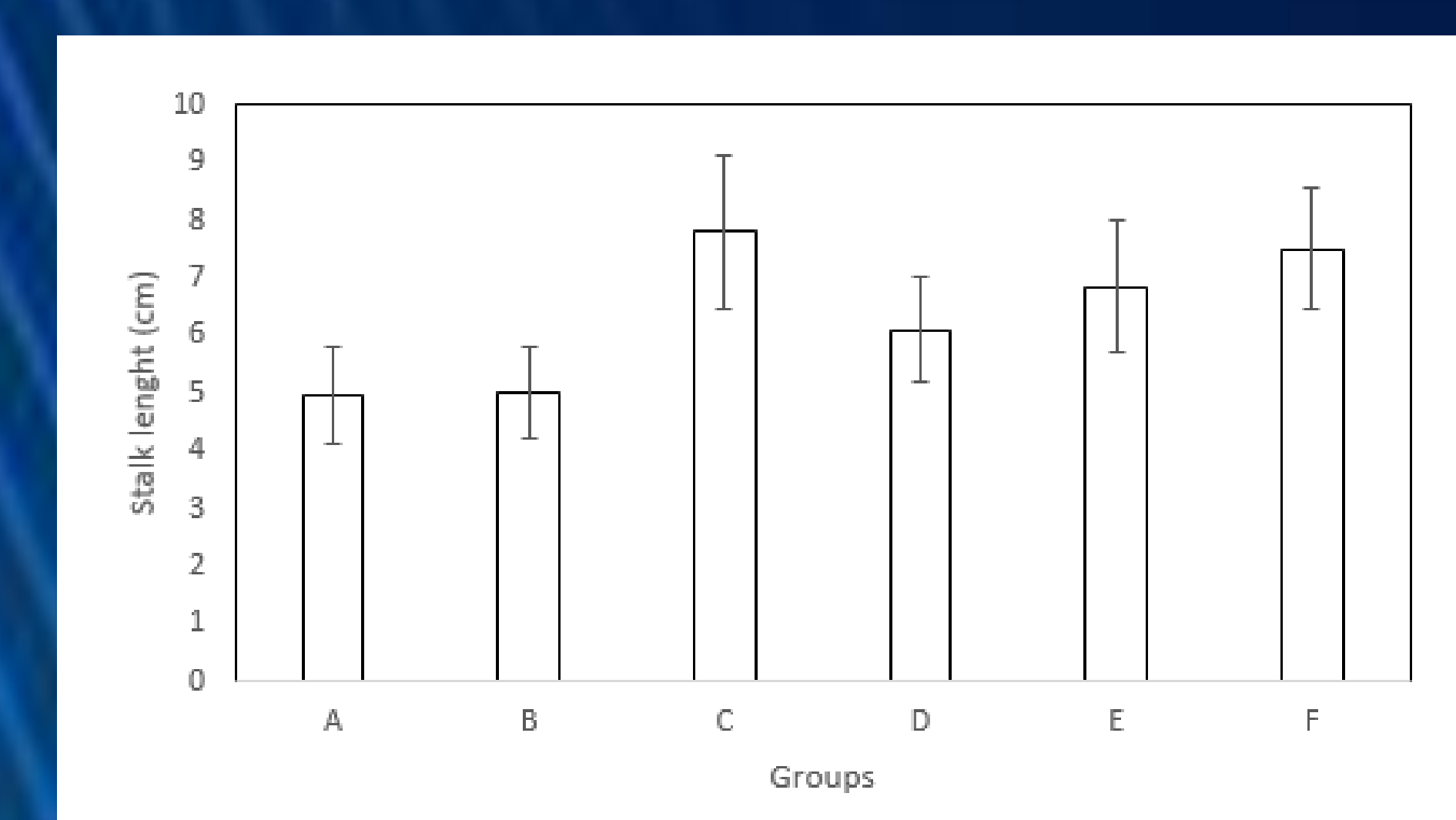
In compliance with the Avogadro number, the potency of 36x used in the present study contains only the molecules of the diluent medium, in this case, water. As showed in the figures 1, 2, and 3, we assumed that growth stimulation of the wheat stalks was produced by influence of diluted water samples of the original substance (fertilizer at 2% used as a positive control in all bioassays) compared with negative controls of water 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 of theoretical 0-molarity of 10-24 (Chikramane, et al., 2010; Seker et al., 2018).



Bioassay 1. Mean stalk lengths of wheat under the influence of three diluted-agitated fertilizer. (A) negative control of untreated water, (B) Sham-treated water, (C) positive control of 2% commercial fertilizer (Stock solution), (D) 7x dilution, (E) 14 x dilution, and (F) 36 x dilution. Bars represent arithmetical mean $\pm$ SD



Bioassay 2. Mean stalk lengths of wheat under the influence of three diluted-agitated fertilizer. (A) negative control of untreated water, (B) Sham-treated water, (C) positive control of 2% commercial fertilizer (Stock solution), (D) 7x dilution, (E) 14 x dilution, and (F) 36 x dilution. Bars represent arithmetical mean $\pm$ SD



Bioassay 3. Mean stalk lengths of wheat under the influence of three diluted-agitated fertilizer. (A) negative control of untreated water, (B) Sham-treated water, (C) positive control of 2% commercial fertilizer (Stock solution), (D) 7x dilution, (E) 14 x dilution, and (F) 36 x dilution. Bars represent arithmetical mean $\pm$ SD

CONCLUSION

All samples of diluted fertilizer used here influenced significantly the stalk growth. These findings would mean that information has been stored in the test sample.

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