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Droplet evaporation method applied to test the efficacy of *Zincum metallicum* 30c on stressed and non-stressed wheat seeds

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Background: The droplet evaporation method (DEM) has been recently proposed as a possible tool to test the efficacy of ultra high dilutions (UHDs) [1]. Here we applied the same methodology consisting in the evaporation of droplets from leakages obtained by placing wheat seeds in UHDs to test whether DEM patterns vary in function of the tested treatment: *Zincum metallicum* 30c (ZM), lactose 30c as dynamized control (L), and unsuccussed, undiluted water as negative control (C). Since our previous study [1] showed that there is a significant increase in the UHD action in the stressed model, with respect to the non-stressed one, also in the present experiment we tested ZM, L and C on both stressed (s-seeds) and non-stressed wheat seeds (ns-seeds). **Aims:** The aim of the experiment was to test whether treatments ZM, L, and C applied on ns- and s-seeds influence the DEM pattern characteristics. **Materials and methods:** Whole, undamaged wheat seeds (*Triticum aestivum* cv. Inallettabile, harvesting year 2010) were used for the experiment, following the experimental protocol described in [1]. The distinction between s- and ns-seeds is based upon different storing conditions from the harvest until the analysis day: the ns-seeds were kept in controlled conditions at 5°C and in the dark, whereas the s-seeds were kept in lab at varying temperature, humidity and light conditions. As far as treatment preparation is concerned, ZM and L were obtained from triturations 3c (received from the Federal University of Rio de Janeiro) by vertical mechanical succussions and following dilutions; negative control (C) was ultra-pure water from the same water batch used for dilutions. The experiment was repeated on 6 days within one month; each seed lot (ns- and s-seeds) was analyzed on 3 different days (s-seeds on 10th, 11th and 24th February 2014 and ns-seeds on 12th, 26th February and 3th March 2014), with three treatment-replicates for ZM and L and two for C, three repetitions for each treatment-replicate and 5 droplets per repetition (360 patterns for each seed lot). The complexity degree of polycrystalline structures obtained from the evaporating droplets was measured by means of the ImageJ software [2] by calculating their local connected fractal dimensions (LCFD). The data on moon phase, moon position and moon distance from Earth at the beginning of each evaporation process were collected from the online tool *virtuelles Telescope* [3]. All data were processed by a three-way ANOVA at a significance level of $p \leq 0.05$. Correlations between the moon data and LCFD were evaluated by r Pearson coefficient. **Results:** The effect of the treatment on LCFD values of DEM patterns was significant only in the stressed model: ZM showed a crystallization inhibiting action *vs*. C on all 3 analysis days, whereas a significant difference between ZM and L could be observed only on first and third analysis day (Fig 1a). ANOVA analysis showed that the overall LCFD means for the s-seed lot differed significantly between each other: 1.33 (a) for C, 1.26 (b) for L, and 1.19 (c) for ZM. Moreover, the LCFD values of both seed lots showed a strong day factor influence. A possible explanation of this finding might be the influence of the moon (position, phase, and distance from Earth) on the crystallization process: strong correlation between the LCFD of the patterns and moon data were found (r values were from -0.72 to -0.97). **Discussion:** The present study confirms a pre-sensitization effect towards UHD action in stressed models: a significant inhibiting effect of ZM 30c was found

in all experimentation day. Furthermore, the strong correlations observed between the LCFD values and moon data indicate that the complexity of polycrystalline structures from evaporating droplets of wheat seed leakages might be affected by tidal forces. Since DEM needs to be standardized, these correlations can be considered only simultaneous and not causal, however their strength gives good reasons for further studies. **Conclusions:** The results of the present pilot-study seem to encourage further DEM experiments on s-seeds following UHD treatments. For further confirmation of the inhibiting effect of ZM on s-seeds, germination tests should be planned. Finally, the performance of DEM experimentations during days and hours with equal tidal influence on gravity might be helpful for the reduction of the day factor.

Keywords: Ultra high dilutions, droplet evaporation method, patterns, *Zincum metallicum*

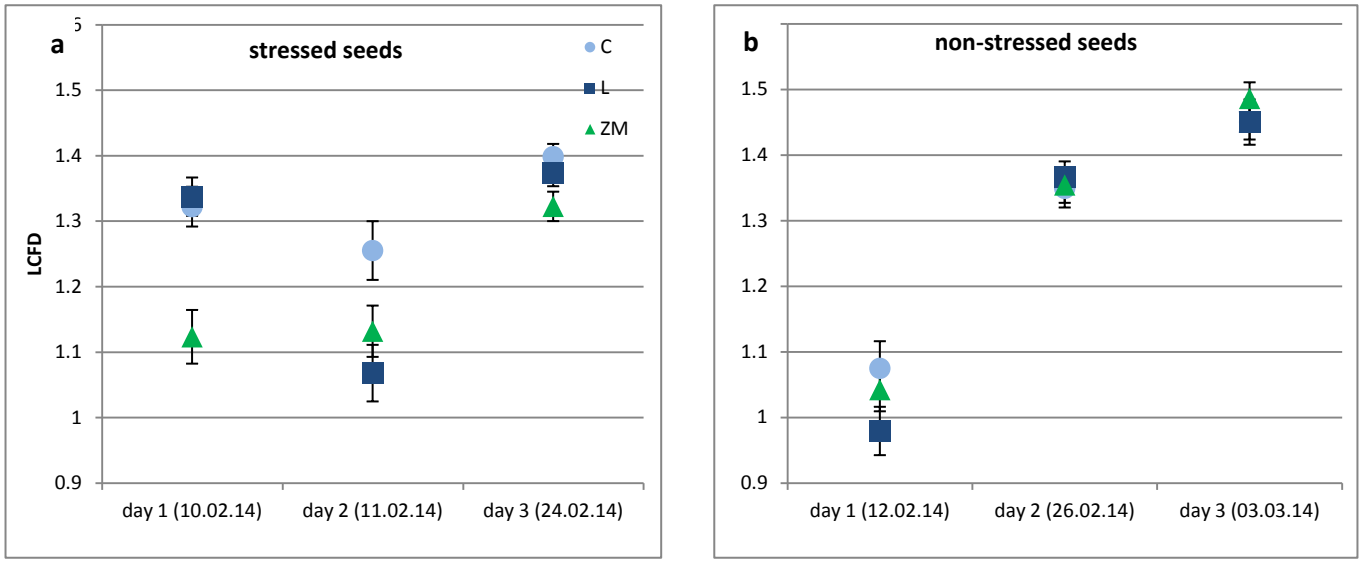

Figure 1: Graphical representation of the mean local connected fractal dimension values (LCFD) of droplet evaporation patterns obtained from stressed and non-stressed wheat seeds following treatments with *Zincum metallicum* 30c (ZM), lactose 30c (L), and unsuccussed/undiluted water (C).

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