Effects of silver nanoparticles on wheat seedlings growth evaluated using ultra-weak photon emissions measurements

Efeitos das nanopartículas de prata no crescimento das plântulas de trigo avaliadas através de medições de emissões de fótons ultra fracos

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ABSTRACT
Ultra-weak photon emission (UWPE) is used to evaluate the effects of silver nanoparticles (AgNP) on wheat seeds germination. Three test series, using wheat seed samples in triplicate, irrigated by three different solutions: AgNP colloid, deionized water and sodium citrate dihydrate solution, had their UWPE data acquired for 24 hours at the fifth day of germination, inside an especially designed dark chamber. The photon-counts summation of each test was calculated, and correlated to its respective germination parameters – total biomass gain and total elongation of seedling aerial part. AgNP tests presented inferior total photon-counts summation and germination parameters levels, as compared to the tests using the other two solutions. Besides, the UWPE temporal profiles

decreased over time for the AgNP tests, while for the other two test solutions the emission increased over time. Those results points changes in physiologic functions as deleterious effects of the AgNP contaminant. Principal Component Analysis (PCA) was applied, and the AgNP tests could be separated of the other tests due to germination parameters and UWPE data. The proposed method using UWPE measurements seems to be feasible to evaluate germination parameters of wheat seed in the presence of AgNP, and potentially in the presence of other contaminants.

Keywords: Silver nanoparticles, Ultra-weak photon emission, Germination tests, Wheat seedling.

RESUMO

A emissão de fótons ultra fracos (UWPE) é utilizada para avaliar os efeitos das nanopartículas de prata (AgNP) na germinação das sementes de trigo. Três séries de testes, utilizando amostras de sementes de trigo em triplicata, irrigadas por três soluções diferentes: O colóide AgNP, água desionizada e solução de citrato de sódio dihidratado, teve seus dados de UWPE adquiridos por 24 horas no quinto dia de germinação, dentro de uma câmara escura especialmente projetada para isso. A soma de fótons de cada teste foi calculada e correlacionada com seus respectivos parâmetros de germinação - ganho total de biomassa e alongamento total da parte aérea das mudas. Os testes AgNP apresentaram níveis inferiores de soma total de fótons e parâmetros de germinação, em comparação com os testes que utilizaram as outras duas soluções. Além disso, os perfis temporais UWPE diminuíram ao longo do tempo para os testes AgNP, enquanto que para as outras duas soluções de teste a emissão aumentou ao longo do tempo. Esses resultados apontam mudanças nas funções fisiológicas como efeitos deletérios do contaminante AgNP. A Análise de Componentes Principais (PCA) foi aplicada, e os testes AgNP puderam ser separados dos outros testes devido aos parâmetros de germinação e dados de UWPE. O método proposto usando medidas de UWPE parece ser viável para avaliar parâmetros de germinação de sementes de trigo na presença de AgNP, e potencialmente na presença de outros contaminantes.

Palavras-chave: Nanopartículas de prata, emissão de fótons Ultra-Weak, testes de germinação, plântulas de trigo.

1 INTRODUCTION

The ever growing demand for quality food is a great concern worldwide as the world population grows and advances in medicine promotes longer life expectancy. In this context, it is crucial to enhance agriculture production, since, grains, together with animal protein, represent a major source of food.

Moreover, germination parameters of seeds may be influenced by many different factors, being one of them a recent phenomenon, which is the stress imposed to seeds by the presence of metallic nanoparticles in the air, soil and water, originated from improper disposal of industrial residuals [1]. The potential action of metallic nanoparticles as antimicrobial was already demonstrated in [2], however, the interaction between seeds and metallic nanoparticles which might happen in many different ways, and the consequences of those interactions are not totally known, which has been attracting much attention from the scientific community in this field [3,4,5].

Important to notice that today´s method applied to evaluate germination parameters are time consuming, requiring specialized personal to be accomplished, and produce more variable than desired [6], so, the proposition of new effective methods applied to the analysis of contaminants affecting germination parameters of seeds is a quite important issue to prevent production loses and to guarantee food safety, especially if the methods proposed bring fast results compared to the traditional method.

The ultra-weak photon emission (UWPE), also known as ultra-weak bioluminescence and biophoton emission, among other terms, is a phenomenon present in all living organisms, associated to the biological metabolism, being characterized as a permanent light emission ranging from the ultraviolet to near-infrared, and having intensities from tens to hundreds photons.cm-2.s-1[7,8]. In order to detect and acquire UWPE data an especially designed apparatus capable of doing photon counting, and based on low dark-noise photomultiplier module, must be designed and assembled [9,10].

UWPE measurements of seeds samples have been studied and used to evaluate germination parameters by many research groups around the world [11,12,13,14,15,16]. In this paper we propose a method to indirectly evaluate germination parameters using UWPE in order to characterize the impact of the presence of silver nanoparticles (AgNP) in wheat seedlings. The proposed method shows to be very promising to evaluate effects of contaminants in wheat seed germination and seedling growth, since it presents some advantages, such as: shorter period of time demanded to accomplish the tests and it is less laborious than traditional methods.

2 MATERIALS AND METHODS

2.1 SEEDS SAMPLES PREPARATION

Each sample test was comprised of 25 wheat seeds (Triticum aestivum) randomly taken from high germination potential of wheat seeds stock, weighted in a precision scale (Pocket Scale®, MH-500), arranged over 5 filter paper substrate on a 8 cm Petri dish (both previously sterilized), and imbibed with 6 mL of one of the three irrigators described later on section 2.2. After a period of 72 h germinating inside a germination chamber in dark, at controlled temperature of 20°C, the sample test received a second imbibition with 2 mL of the same irrigator at the fourth day, and after a total period of 96 h after seeding. Then, immediately after the sample left of germination chamber, it was transferred in low light condition to the dark chamber of the photon counting apparatus to perform the UWPE data acquisition for 24 h. The tests for each irrigator were done in triplicate.

2.2 IRRIGATORS PREPARATION

The irrigators used for the UWPE tests were of three types: deionized water (1), sodium citrate dihydrate solution (2) and silver nanoparticles colloid (3). Solution (1) was comprised only by deionized water (Cloroquímica®, pH 7.0, conductivity 5 µS/cm). Solution (2) is comprised 9.99 mL of deionized water and 0.01 mL of sodium citrate dihydrate (BIOTEC®, P.A. 99.9 %) with final molar concentration 0.01 mM. Solution (3) was an AgNps colloid synthesized by pulsed laser ablation in liquids (LAL) from a silver plate (CAS number 7440-22-4, thickness of 1.0 mm, 99.9% trace metal basis, Sigma Aldrich®). A Nd:YAG laser (Tempest-20, New Wave Research®, wavelength 532 nm, 5 ns pulse, 5 mm beam diameter, Fremont, CA, USA) was focalized by a 15 cm focal length on the silver target positioned at the bottom of a Becker with 10 mL of solution 2. The energy per pulse, pulse rate, the ablation time was adjusted to 17 mJ, 10 Hz and 20 minutes, respectively. The extinction spectra of colloid was measured with a UV-Vis optical fiber spectrometer (HR4000, Ocean Optics®, 200 – 1100 nm spectral range, composite grating HC-1) and using a tungsten halogen light source (LS-1, Ocean Optics®, Dunedin, FL, USA). The extinction spectrum was recorded using an integration time of 5 ms and 100 averages. The above mentioned procedure resulted in a silver nanoparticles colloid with its intensity of the extinction band at 400 nm and optical density at 1.01 arbitrary units. Moreover, AgNPs colloid was synthetized the same day as the start of each test. And, the solution (2) was used to ensure the stability of AgNps colloid. So, it was necessary to perform UWPE tests with only the solution (2). In summary, the tests with solution (2) were carried out with the objective of analyzing that the influences found in the tests with solution (3) were referring the AgNps colloid.

2.3 GERMINATION ANALYSIS

Once the UWPE data acquisition finished, at the end of the fifth day, the seeds samples were dried from the residual water with paper towel, weighted again to measure their biomass gain, and then their length of seedling aerial part measured and totalized.

2.4 UWPE DATA ACQUISITION

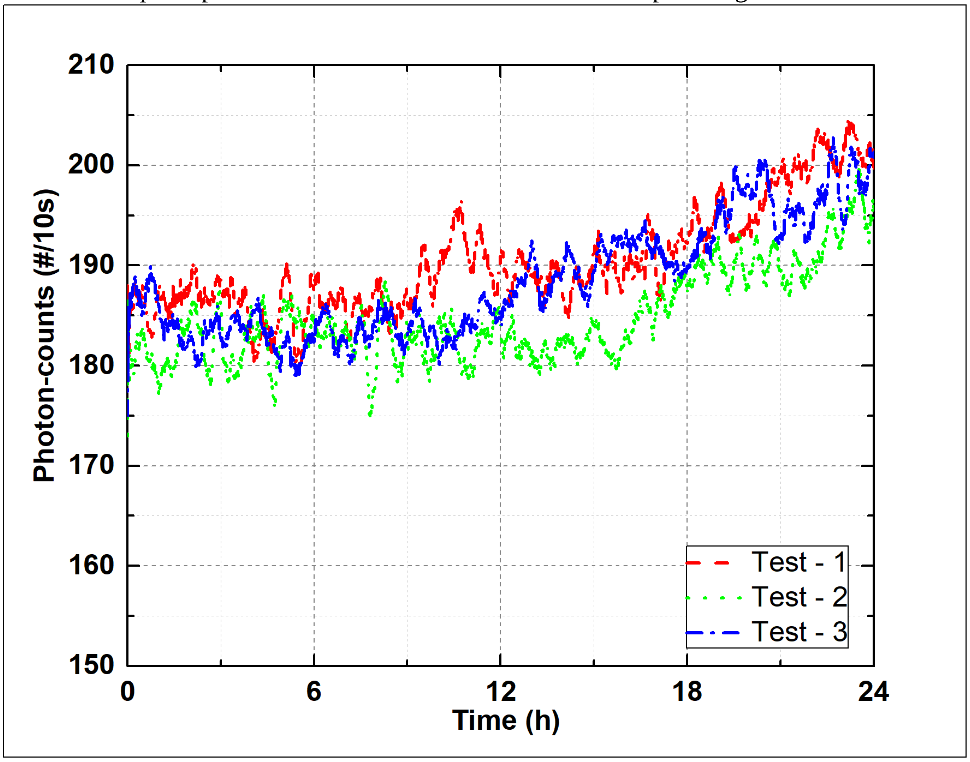
Photon-counting measurements of the seeds samples were performed using a especially designed apparatus presented by Bertogna et al. [10], provided with a dark chamber, photomultiplier module with low dark count noise and a virtual instrumentation to transfer the acquired UWPE data to a personal computer. Dark noise of the dark chamber was evaluated, resulting an average noise was 163 photons/10s. The UWPE data acquisition period for all tests was 24 h after an initial germination period of 96 h, as already described in section 2.1..The gate timing used to integrate the counts was 10 s. The interior of the dark chamber, where the Petri dish was standing, was maintained at 20°C by a temperature controller. The photon-counting data after being transferred to the personal computer was smoothed by local averaging of 100 adjacent counts in order to minimize noise variance. Also, the total photon-counting summation was calculated as a test parameter. Furthermore, Principal Component Analysis (PCA) technique was applied to discriminate between germination parameters of seeds irrigated by the three different irrigators. Thus, The PCA used the following parameters: summation of UWE, biomass gain and length of aerial part of each test.

3 RESULTS AND DISCUSSION

The results of the three experimental series, using the three irrigators, are presented here: solution (1) - deionized water, solution (2) - sodium citrate dihydrate, solution (3) – silver nanoparticles colloid.

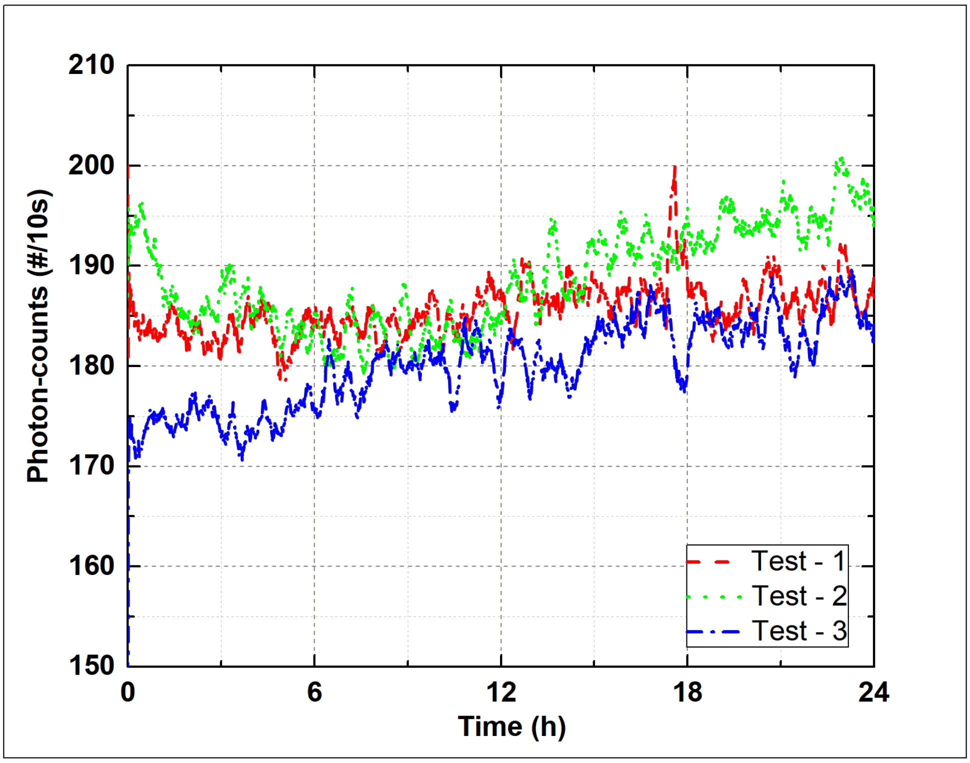
Fig. 1 presents the UWPE temporal profiles obtained from the UWPE measurement tests using seed samples irrigated with solution (1) - deionized water. The plots show a strong similarity in the UWPE temporal profiles among the three tests with an increasing photon-counting over time as the seeds continue to germinate inside the dark chamber. This behavior reflects the increasing in metabolism as the seedlings were growing and gaining biomass.

Fig. 1. UWPE temporal profiles for the tests with wheat seeds samples irrigated with deionized water.



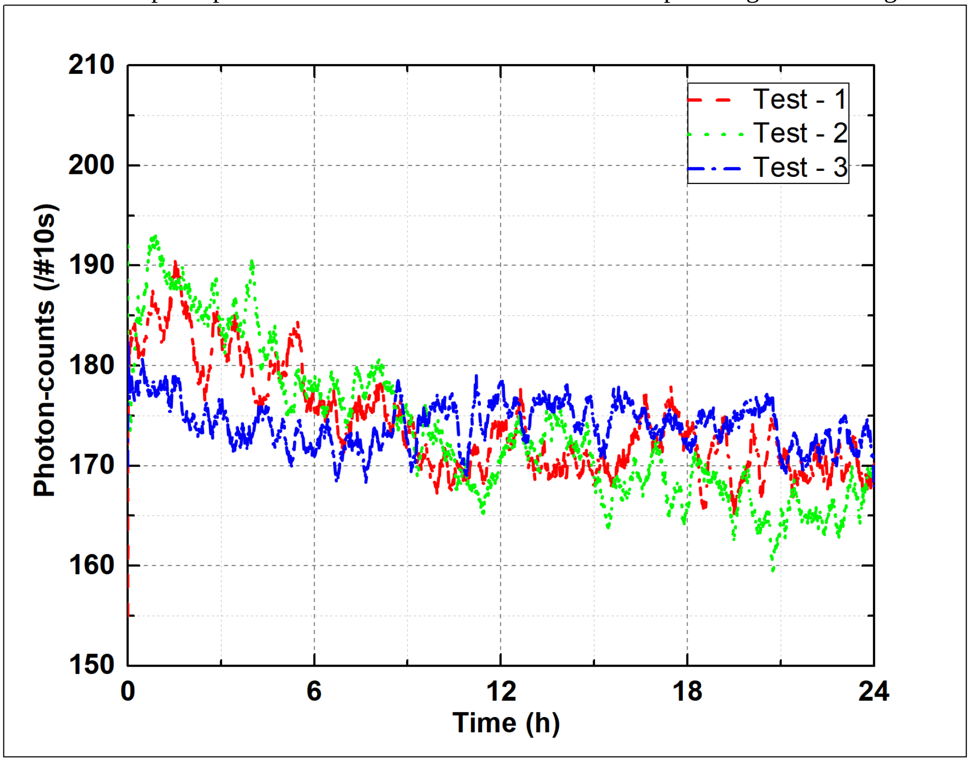
In Fig. 2 the UWPE temporal profiles obtained from the seeds samples using the solution (2) as irrigator - sodium citrate dihydrate solution. Those UWPE temporal profiles had shown a similar behavior among the three tests, as seen from the deionized water tests, that is, presenting an increased photon-counting over time. Once again, this behavior reflects the increasing in metabolism as the seedlings were growing and increasing their biomass. Those results are supported by the fact that both solutions (1) and (2) do not affect the metabolism of the seeds, being both innocuous in terms of disturbances in the seeds metabolism, being the biomass gain results from both test series very close to each other, as shown in Table 1. Also shown in Table 1 are the total photon-counts for all test series for the UWPE measurement period of 24 h, being those data a subsidiary parameter that indicate the metabolism level of seedlings growth.

Fig. 2. UWPE temporal profiles for the tests with wheat seeds samples irrigated with sodium citrate dihydrate solution.



Finally, in Fig. 3 are presented the 24 h UWPE temporal profiles for the tests using solution (3) as irrigator to the seeds samples – AgNP colloid.

Fig. 3. UWPE temporal profiles for the tests with wheat seeds samples irrigated with AgNP colloid.



It can be noticed in Fig. 3 that, when compared to the two UWPE test series previously presented in Fig. 1 and Fig. 2, the temporal profiles presented by the samples irrigated with AgNP colloid have shown a distinct behavior. Those UWPE temporal profiles for AgNP irrigator had a decreasing photon-counting trend over time, being that behavior associated with a decreasing in metabolism as the seedlings were growing, which led to biomass gain inferior compared to the two other test series. It is important to realize that the influence of AgNPs colloids can be start before of the last 24 h of test. But, through of analyses, it noticed that the UWPE temporal profiles of different irrigation it was more evident in this period.

In Table 1, the germination parameters for all samples, and for all irrigators, are presented as the total biomass gain after 120 h of seeding and the total photon-counts in 24 h from the UWPE tests. Those results support the fact already discussed about the trend in the UWPE temporal profiles to increase in tests series using solutions (1) and (2), and the trend in UWPE temporal profile to decrease in tests series using solution (3) – AgNP colloid.

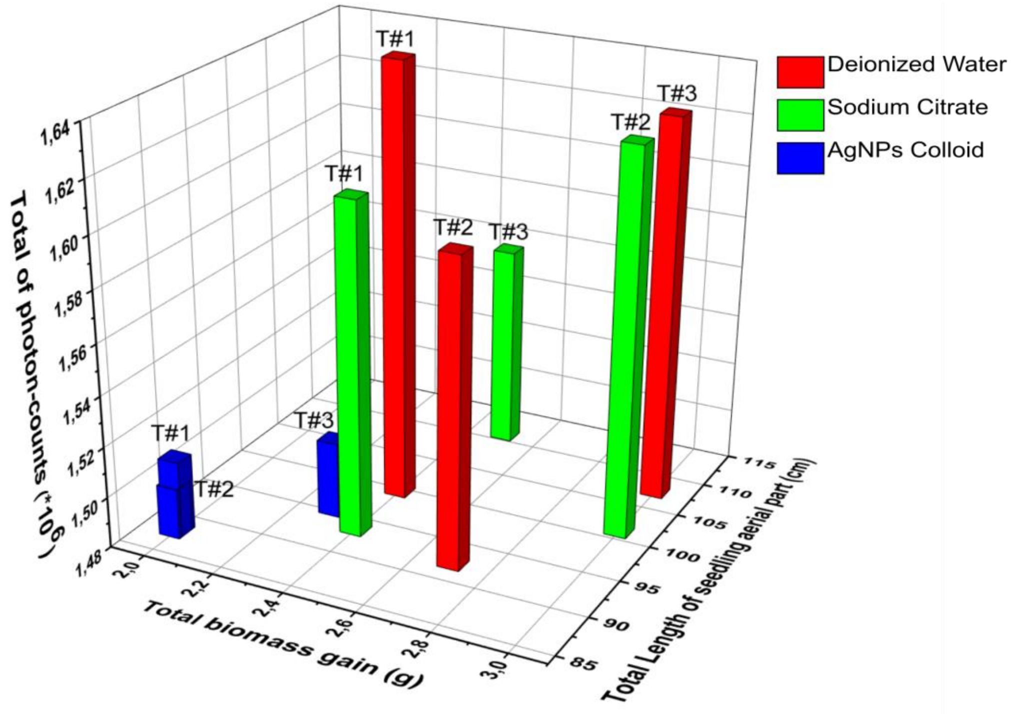
Table 1. Germination parameters represented by the seeds samples total biomass gain and length of seedlings aerial part after 120 h and total photon-counts for the three irrigators.

Irrigator type	Test	Total biomass gain (g)	Total length of seedling aerial part (cm)	Total photon-counts * 10^6
Deionized water	1	2.4	99.60	1.65
	2	2.7	92.20	1.60
	3	3.0	107.50	1.63
	Avg.	2.7	99.80	1.63
Sodium citrate dihydrate	1	2.4	93.40	1.60
	2	3.0	101.10	1.63
	3	2.5	110.90	1.65
	Avg.	2.6	101.80	1.63
AgNP colloid	1	2.0	88.00	1.51
	2	2.0	87.70	1.50
	3	2.3	95.10	1.51
	Avg.	2.1	90.30	1.51

In the last test series, for the AgNP colloid, it can be noticed a disturbance in the seeds metabolism, when compared to the other two irrigators, proved by the inferior gain of biomass with an average of 2.1 g and the decrease levels of lengths of seedlings aerial parts with an average 90.3 cm. Those results related to the other two irrigators, which are deionized water and sodium citrate dihydrate solution, showed an average gain of biomass of 2.7 g and 2.6 g, respectively. Besides that, lengths of seedlings aerial parts for those irrigators had an average of 99.8 cm and 101.8 cm, respectively, considerably greater than the value presented by AgNP irrigator.

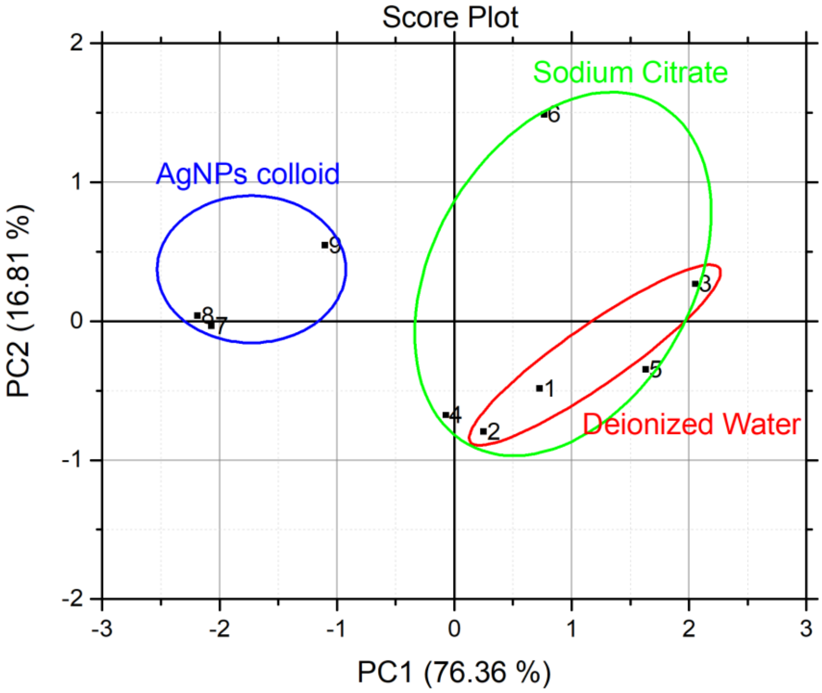
In Fig. 4, the total photon-counts of the UWPE temporal profiles from the three series of tests, as well the germination parameters represented by the total biomass gains and lengths of seedlings aerial parts are presented altogether in a 3-D plot.

Fig. 4. UWPE total photon-counts versus gain of biomass versus length of seedling aerial part for all three test series.



In Fig. 5 represents the scores of the first component (PC1) versus the scores of the second component (PC2).

Fig. 5. Scores for the first component (PC1) versus the scores of the second component (PC2) of the tests.



It can be noted that the first component explained 76.36% of the data variability, while the second explains 16.81%. It is interesting to note the arrangement of the nine tests over PC1. The tests with samples irrigated with AgNP colloid had an opposite sign when compared to tests with samples irrigated with deionized water and sodium citrate dihydrate. The test 2 of samples irrigated with the AgNP colloid had the highest weight, contributing more to PC1, reflecting the greater variability of this test, when compared to the other tests. Thus, there are reasons to believe that the PC1 models the behavior of the tests performed. In addition, through the PCA, it was possible to verify that the tests with samples irrigated with the AgNP colloid are grouped together, but separate from the other tests, showing different germination parameters.

4 CONCLUSIONS

The UWPE temporal profiles acquired from wheat seeds samples irrigated by three different solutions – deionized water, sodium citrate dihydrate and AgNP colloid, led to the same conclusions, that is, the nanoparticles colloid a special designed UWPE measurement in dark chamber, applied to impose a stress condition to the seeds samples causing a depression on the seeds metabolism. And, maybe, lower levels of biomolecules synthesis, pointed also by the decreasing UWPE profiles over time. Besides that, the tests presented less biomass gains and lengths of seedlings aerial parts when compared to the two other solutions. Those results are a clear indication that silver nanoparticles colloid when participating in the germination process can decrease the germination parameters of wheat seeds and potentially cause economic losses to agriculture.

The method here presented to evaluate wheat seeds germination parameters, based on the UWPE measurements, shown to be a very effective way to evaluate the germination parameters of seeds in general, and may be applied to other stressing irrigators than nanoparticles colloid, commonly present in nature as improper disposal of byproducts of industrial production.

It is worth mentioning that, the results presented are valid for the lots used in these test, and do not represent averages for different cultivars.

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