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Original paper

Fe_3O_4 – water based magnetic nanofluid influence on weight loss of wheat seedlings under controlled conditions

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Abstract Different species of nanoparticles are of special interest through the perspective of nano-biotechnologies in agriculture as they can be used for controlling plant growth, effective use of nutrients and plant resistance to various stresses factors. The present studies explored the effect of a biocompatible aqueous magnetic nanofluid (MNF) with Fe_3O_4 nanoparticles on wheat seedling, Triticum aestivum L. ssp. vulgare, Alex cultivar. The magnetic nanofluid was added in different concentrations (0.0%, 0.05%, 0.1%, 0.5% and 1%) for obtaining water solutions which were used for treating wheat grains by imbibition. Wheat seedlings, aged 10 days, were analyzed in terms of biomass accumulation, soluble carbohydrate content and rate of weight loss by drying under controlled conditions. The parameters analyzed describe the total drying time (T_t), the drying time needed for reaching the maximum weight loss rate (t_m), medium rate (RWL_{avg}) and maximum rate (RWL_{max}) of weight loss. Total drying time was higher ($p<0.01$) in the variants treated with MNF ($1515.00\pm45^{**}$ - $1725.00\pm25^{***}$ seconds) than in the control variant (1380.00±60 seconds). RWL_{max} was lower under the influence of MNF (0.336 – 0.432 g/min) compared to the control variant (0.462 g/min), $p<0.01$.

Keywords Controlled drying, magnetic nanoparticles, water loss, weight loss rate, wheat seedlings

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Introduction

Numerous studies have recently been run on the relation of different species of nanoparticles (Cd, Co, Au, Zn, Ag, Al, TiO_2, Si) with various biotic or abiotic media (DOSHI & al. 2008 [1]; SHARMA & al. 2009 [2]; NARAYANAN and SAKTHIVEL 2011 [3]; ZHANG & al. 2011 [4]). In addition to the already conventional nanoparticles, recent research has focused on other nano structures based on carbon (KHODAKOVSKAYA & al. 2009 [5]), rare earth oxide nanoparticles (MA & al. 2010a [6]) or engineered nanoparticles – ENPs (MA & al. 2010b [7]).

Many studies have analyzed the nanoparticle potential for ecotoxicity, phytotoxicity and genotoxicity on various biological media, revealing certain types of toxicity in relation to the species of nanoparticle and the reference medium analyzed (RĂCUCIU and CREANGĂ 2007a [8]; LIN and XING 2007 [9]; BARRENA & al. 2009 [10]; GHOSH & al. 2010 [11]; KUMARI & al. 2011 [12]; ELSAESSER and HOWARD 2012 [13]).

It is already known that nanoparticles of different species are absorbed by plants, integrated into the transport network of conductive vessels with the possibility to accumulate in the structure of some tissues. In this respect, many studies have proven that a series of nanoparticle species can penetrate the biological barriers of vegetal cells (LIN and XING 2008 [14]; LIN & al. 2009 [15]; WILD and JONES 2009 [16]; CIFUENTES & al. 2010 [17]; CORREDOR & al. 2010 [18]).

NAVARRO & al. (2012) [19] investigated the uptake of water-dispersible CdSe/ZnS quantum dot nanoparticles by *Arabidopsis thaliana* L. Heynh. plants. Other studies have followed the influence of TiO_2 nanoparticles in different concentration in *Triticum aestivum* L. spp. (LARUE & al. 2012 [20]) and in *Ulmus elongate* L.K.Fu. & C.S. Ding (GAO & al. 2013 [21]). KOELMEL & al. (2013) [22] investigated gold nanoparticles uptake and their tissue level distribution in *Oryza sativa* L. plants. POKHREL and DUBEY (2013) [23] reported the developmental responses of two agriculturally significant crop plants, *Zea mays* L. and *Brassica oleracea* var. *capitata* L., upon in vitro exposure to nanoparticles of citrate-coated silver (Citrate–nAg) and zinc oxide (nZnO).

Agriculture is one of the fields of interest for nanomaterials and nano-technologies. Different studies and research have appreciated the potential and perspectives of nano-biotechnologies in plant nutrition and protection, by directional control of agrochemical substances or macromolecules for controlled seed germination, plant growth or for improved plant disease resistance, efficient nutrient use and pathogen detection (NAIR & al. 2010 [24]; CHEN and YADA 2011 [25]; KHOT & al. 2012 [26]).

Magnetic nanoparticles, Fe_3O_4 type, based on specific properties also show interest in this direction. Some studies have observed the influence of these nanoparticles species, formulated as a magnetic liquid, on morpho-anatomical and physiological parameters in different species (SALA 1999 [27]; RĂCUCIU and CREANGA 2007b [28]; ZHU & al. 2008 [29]; PIRVULESCU and SALA 2012 [30], 2013 [31]; PÎRVULESCU & al. 2015 [32]). No bibliographical references have been found on the influence of magnetic nanoparticles and magnetic nanofluids on the water regime in vegetal organisms and especially under controlled thermal conditions.

The present study focuses on the influence of Fe_3O_4 – based magnetic nanofluids on the water regime in wheat seedlings, under controlled drying conditions, as wheat has high ecological plasticity and is used in many studies in relation to different species of nanoparticles.

Materials and methods

Magnetic nanofluid used. The present research tested the influence of Fe_3O_4 magnetic nanoparticles conditioned as biocompatible magnetic nanofluid (VEKAS & al. 2009 [33]). The magnetite nanoparticles were synthesized by chemical coprecipitation and stably dispersed in water carrier by double layer sterical stabilization with oleic acid (OA+OA) (BICA & al. 2007 [34]). The aqueous magnetic nanofluid MNF/H_2O (OA-OA) was characterized by saturation magnetization 32Gs and density $\rho_{25^oC} = 1.0201$ g/cm^3. The volume fraction of magnetite nanoparticles was $\varphi = 0.005$. Concerning toxicity aspects, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have already approved the medical use of magnetic iron oxides MNPs which include magnetite (mixed Fe^{2+} and Fe^{3+} ions), and maghemite (COLOMBO & al. 2012 [35]). Oleic acid used for sterical stabilization of magnetite nanoparticles is the main component of vegetable oils (CANO & al. 2012 [36]; WILSON & al. 2012 [37]; GHAZANFARI & al. 2016 [38]).

Experimental design. The magnetic nanofluid was used in the making of aqueous solutions in five different concentrations: MNF 0% (V_1 - control variant), MNF 0.05% (V_2), MNF 0.1% (V_3), MNF 0.5% (V_4) and MNF 1% (V_5). The biological material was represented by *Triticum aestivum* L. *ssp vulgare* species, *Alex* cultivar. The wheat seeds were treated by imbibitions in the magnetic

nanofluid solutions (variants $V_2 - V_5$) and in water as the control variant, respectively (V_1). The experimental design was monofactorial, arranged in four replications, each of 40 seeds in each variant and repetition. The seeds have a high degree of uniformity, with an average of seed per variant and repetition of 2.188 ± 0.015 g.

Treatment procedure and monitored parameters. Seed treatment was accomplished through imbibition for 15 minutes, in order to facilitate absorbance of solutions in the tegument of wheat caryopses. Penetration of magnetite particles in the tegument of wheat caryopses was not investigated. After imbibition time, wheat grains were places in germination pots on filter paper, in a germination chamber with uniform conditions. Periodically, water in equal quantities was added to the pots. Ten days after germination, 20 seedlings of wheat, harvested randomly from each variant and repetition, were subjected to controlled drying process. Some parameters were determined to characterize the quantity of biomass and weight loss under the influence of magnetic nanofluid: fresh matter gained (Fm - g), dry matter (Dm - g), moisture (M - %), carbohydrate content (CHO – mg/100g fresh matter), average weight loss rate (RWL_{avg} - g/min), maximum weight loss rate (RWL_{max} - g/min), total drying time (T_t - s), drying time until reaching the maximum weight loss rate (t_m - s). The determinations regarding the drying parameters and the moisture parameters were made with a thermo balance with a precision of ±0.001 g (AXIS Thermobalance, Model ATS 60, Poland). The drying process took place at 100° C, with automatic stop at the moment when five consecutive readings gave a minimal difference, in order to let each sample reach complete drying. Between 143 and 175 data recordings were made regarding the drying parameters, at 10 seconds intervals. Reductive carbohydrates (glucose) were determined via the enzymatic method, using Cayman colorimetric assay kit. This assay use different enzymatic redox reactions which finally producing hydrogen peroxide. This reactive compound reacts witch 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to generate a pink dye with an optimal absorption between 500-510 nm. Glucose standard solutions were prepared in the 3-30 mg/L concentrations range. 850µL diluted Assay Buffer and 150µL of samples (standards) were used. The reactions were initiated by adding 500 µL of enzyme mixture to all standards and samples. Incubate the test tubes for 10 minutes at 37 °C and after read the absorbance at λ = 505 nm using a spectrophotometer (Specord 205, Analytik Jena, Germany). Dilute samples with distilled water if necessary. The concentrations were interpreted using the calibration curve.

Statistical methods of analysis. The experimental data of wheat plantlet parameters were processed through analysis of variance (ANOVA) using the mathematical module on EXCEL 2007. Correlation coefficient and regression analysis were performed using the PAST software (HAMMER & al. 2001 [39]). The differences between the means were compared using LSD values; $p < 0.5$ ($LSD_{5\%}$), $p < 0.1$ ($LSD_{1\%}$), $p < 0.01$ ($LSD_{0.1\%}$).

Results and discussion

Wheat seeds imbibed with magnetic nanofluid solutions in different concentrations ($V_2 - V_5$), and with water respectively (V_1), germinated and led to the formation of seedlings that evolved in uniform growth conditions. The quantity of fresh matter gained by wheat seedlings under the influence of magnetic nanofluid (variants $V_2 - V_5$) ranged from 3.491±0.02 g to 4.002±0.10 g, the higher values being recorded with low concentrations of magnetic nanofluid. In the control variant, 3.683±0.14 g fresh matter was recorded. The dry matter resulted from the controlled drying of the fresh matter, with real time monitoring of the weight loss, presented obvious differences between the variants treated with magnetic liquid and the control variant, as shown in Table 1. In the control variant, dry matter values were 0.415±0.014 g, while in the variants treated with magnetic nanofluid the values ranged from 0.399±0.005 to 0.467±0.027 g. Positive correlation was found between dry matter and fresh matter (r = 0.977), while the correlation was negative with sample moisture (r = -0.711) and with carbohydrate content of wheat seedlings, respectively (r = -0.556). Wheat seedling moisture ranged from 88.29±0.33 to 88.67±0.24% in the variants treated with nanomagnetic fluid and 88.74±0.06 % in the control variant. The differences identified presented statistical assurance only with variant V_2 (MNF 0.05%). Positive correlation was identified between the water content and the carbohydrate content of wheat seedlings (r = 0.960). The carbohydrate content (CHO) in wheat seedlings oscillated between 4.514±0.11 and 5.902±0.18 mg/100 g fresh matter in the variants treated with magnetic nanofluid and 6.147±0.15 mg/100 g fresh matter in the control variant. A decrease was found in the carbohydrate content of wheat seedlings sprung from seeds treated with magnetic nanofluid, when compared to the control variant, with statistical assurance of the differences ($p<0.01$).

Table 1. Experimental data regarding the influence of magnetic liquids on some physiological parameters of wheat seedlings

Variant	Fm (g)	Dm (g)	M (%)	CHO mg/100g dry matter	T_t (s)
V_1 control	3.683±0.140	0.415±0.014	88.74±0.06	6.147±0.15	1380.00±60
V_2	4.002±0.100*	0.467±0.027*	88.29±0.33^{o}	4.514±0.11^{ooo}	1520.00±20**
V_3	3.491±0.020	0.399±0.005	88.57±0.09	5.191±0.29^{ooo}	1535.00±25**
V_4	3.821±0.030	0.433±0.006	88.67±0.24	5.902±0.18	1725.00±25***
V_5	3.696±0.160	0.426±0.037	88.50±0.49	5.400±0.13^{oo}	1515.00±45**
	$LSD^{6}_{5\%}$ = 0.221	$LSD_{5\%}$ = 0.038	$LSD_{5\%}$ = 0.337	$LSD_{5\%}$ = 0.393	$LSD_{5\%}$ = 72.01
	$LSD_{1\%}$ = 0.322	$LSD_{1\%}$ = 0.055	$LSD_{1\%}$ = 0.491	$LSD_{1\%}$ = 0.572	$LSD_{1\%}$ = 104.7
	$LSD_{0.1\%}$ = 0.48	$LSD_{0.1\%}$ = 0.08	$LSD_{0.1\%}$ = 0.74	$LSD_{0.1\%}$ = 0.86	$LSD_{0.1\%}$=157.1

Fm – fresh matter in wheat leaves; Dm – dry matter in wheat leaves; M – moisture in wheat leaves; CHO – soluble carbohydrate content in wheat leaves; T_t – total sample drying time; LSD – the limits significance of differences. Data represent means ± standard error. Upper-case symbols indicate significance of differences within experimental results; *, **, *** represent the significance of positive differences and ^{o}, ^{oo}, ^{ooo} represent the significance of negative differences for $LSD_{5\%}$ ($p < 0.5$), $LSD_{1\%}$ ($p < 0.1$) and $LSD_{0.1\%}$ ($p < 0.01$), respectively.

Table 2. ANOVA statistical-mathematical analysis: Single factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	10660321	12	888360.1	724.0764	2.26E-53	3.412836
Within Groups	63798.14	52	1226.887			
Total	10724119	64				

Alpha = 0.001

The drying time for fresh biomass recorded in the control variant was 1380.00±60 s, while in all variants treated with magnetic nanofluid the sample drying time was longer ($p<0.01$), with values between 1515.00±45** and 1725.00±25*** s, with statistically significant differences ($p<0.01$), Table 1. Negative correlation was identified between the drying time and average weight loss rate (RWL_{avg}), r = - 0.762. The calculated average weight loss rate (RWL_{avg}) was 0.136±0.011 g/min in the control variant and lower in the variants treated with magnetic liquid (0.113±0.001 g/min in V_4 – MNF 0.5%).

Anova single factor statistical analysis revealed that the experimental results as a whole presented high statistical significance, $p<<0.001$; Fcalc > Fcrit, for Alpha = 0.001 (Table 2).

For efficient water management in the vegetal organism, depending on the environment, nutrition and other factors of influence, over time plants have developed a number of strategies by morphological and anatomic adaptations, biochemical mechanisms for dissipating the excess of radiant energy, adjusting the intensity of photosynthesis through settings of the stomata, accumulating high concentrations of soluble carbohydrates in young leaves and various other mechanisms - enzymatic and non-enzymatic, phytohormones and gene expression (CORNIC and FRESNEAU 2002 [40]; LOWLOR 2002 [41]; COMINELLI & al. 2005 [42]; LIANG & al. 2005 [43]; CAO & al. 2013 [44]).

Many other studies have followed the loss of water and drying regime on plants, fruits and seeds depending on various factors (CURSI and CICERO 2014 [45]; DARVISHI & al. 2014 [46]). Drying kinetics has been analyzed in different plants species and were developed certain mathematical models that described the weight loss or water loss in different plant structures (COSTA SANTOS & al. 2013 [47]; URIBE & al. 2014 [48]). Were identified interdependencies between drying rates, air-drying temperature and some fruit quality indices, such as antioxidant activity in *Physalis Peruviana* L. (LÓPEZ & al. 2013 [49]). At the same time, plants tolerance to water stress and heat is an important element in plant breeding processes (ABBASI & al. 2014 [50]; SAREEN & al., 2014

[51]). All of these issues, and many others, have made that plants water regime to be much studied.

Most of the research studied the content and water regime in plants through the analysis of leaves, seedlings, fruits or of plants as whole organisms, in order to find the influence of stomata, cuticles, epidermis or other internal or external factors on water loss through evaporation and transpiration. These studies used excised leaves, in order to expose tissues and cells directly to the process of controlled drying, for the purpose of eliminating the well-known influence of epidermis, cuticle or stomata. Therefore, the different weight loss rate was influenced by other factors, in the present case the magnetic nanofluid being the induced controlled factor which differentiated the variants and the results obtained.

The results obtained revealed differences among the variants in respect to the values of some parameters: average weight loss rate (RWL_{avg}), maximum weight loss rate (RWL_{max}), time to reach RWL_{max} (t_m) and total drying time (T_t). Positive correlation was identified between the carbohydrate content and the water content in plants (r = 0.960), and at the same time negative correlation between the carbohydrate content and the dry matter in plants (r = -0.556).

During controlled drying, the average weight loss rate (RWL_{avg}), as a result of water loss, presented accelerate increase until reaching a maximum value (RWL_{max}) at a certain variable moment, t_m. Sample weight loss through water evaporation continued, with decreased weight loss rate, until the drying was complete. In the control variant, the maximum weight loss rate (RWL_{max}) was reached 2.20 min after the drying process was initiated, while in the variants treated with magnetic fluid, t_m time, when the maximum value RWL_{max} was recorded, was shorter, 1.10 – 1.40 min. The moisture recorded at moment t_m corresponding to RWL_{max} was also different from one variant to another, having values of 18% in the control variant (V_1) and between 5.96 and 12.03% in the variants treated with magnetic nanofluid ($V_2 – V_5$).

The maximum weight loss rate (RWL_{max}) has different values in the identified t_m moments. In the control variant, RWL_{max} was 0.462 g/min, while in variants treated with magnetic nanofluid, RWL_{max} values ranged from 0.336 to 0.432 g/min, as shown in Table 3.

Magnetic nanofluid influenced the process of water loss in the vegetal matter and determined a decrease of the weight loss rate by controlled drying, the phenomenon being proportional with the nanofluid concentration up to the value of 0.5%.

The weight loss rate in the interval $t_0 – t_m$ until reaching RWL_{max}, expressed in relation to time, RWL = f(t), is best described by third degree polynomial functions, with high statistical certainty ($R^2 = 0.999$; $p << 0.01$), equations 1 – 5, with graphical representation in Figure 1.

$$RWL_{Mt} = -1.895E-07t^3 + 2.307E-05t^2 + 0.00391t - 0.02289 \quad (1)$$

$$RWL_{V2} = -1.177E-06t^3 + 0.0001593t^2 - 0.000264t + 0.01195 \quad (2)$$

$$RWL_{V3} = -5.769E-07t^3 + 7.315E-05t^2 + 0.002835t - 0.0098 \quad (3)$$

$$RWL_{V4} = -2.667E-06t^3 + 0.0003014t^2 - 0.00379t + 0.04114 \quad (4)$$

$$RWL_{V5} = -1.182E-06t^3 + 0.0001503t^2 - 0.0003636t + 0.009429 \quad (5)$$

where: RWL – Rate of Weight Loss (g/min); t – Time (sec).

Table 3. Data on the maximum weight loss (RWL_{max}) in relation to the treatment with magnetic nanofluid

Variant	MNF	Mag	Values at the moment of RWL_{max}			
			t_m	RWL_{max}	M	W
	(%)	(mg/cm^3)	(min)	(g/min)	(%)	(g)
V_1 – Mt	0	0	2.20	0.462	19.00	2.870
V_2	0.05	0.051005	1.30	0.420	8.75	3.744
V_3	0.1	0.10201	1.40	0.432	12.03	3.086
V_4	0.5	0.51005	1.10	0.336	5.96	3.567
V_5	1	1.0201	1.20	0.396	7.51	3.569

MNF – magnetic nanofluid on experimental variants, volume concentration; Mag – magnetite in solution; Values calculated based on the average of experimental data; t_m – time to RWL_{max}; RWL_{max} – maximum weight loss rate; M – moisture; W – weight.

The differences identified between moments t_m when RWL_{max} was reached in the experimental variants treated with magnetic nanofluid and the control variant, ranged from 0.40 to 1.10 min. Analysis of experimental results by mathematical calculations (derivation of equations 1 – 5) proved different periods needed for reaching RWL_{max} in wheat seedlings, in relation to the experimental variants.

Magnetic nanofluid influenced the duration of water loss from wheat seedlings, under controlled drying, the interdependence relation being described by equation (6) for total drying time (T_t) with high statistical certainty ($R^2 = 0.929$; $p = 0.071$) and equation (7) for the drying time required for reaching RWL_{max} (t_m) with lower correlation and statistical certainty ($R^2 = 0.567$; $p<0.433$).

$$T_t = -18.621x^2 + 19.91x + 24.897 \quad (6)$$

$$t_m = 2.1128x^2 - 2.6741x + 1.789 \quad (7)$$

where: T_t – total drying time; t_m– drying time required for reaching RWL_{max}; MNF – magnetic nanofluid concentration.

In addition, magnetic nanofluid influenced, in different proportions, the values for the average weight loss rate (RWL_{avg}) and the maximum weight loss rate (RWL_{max}). The influence of magnetic nanofluid on the values of RWL_{avg}, assessed for the entire drying period, was lower ($R^2 = 0.608$; $p = 0.371$) and the dependence relation is described by equation (8).

The influence of magnetic nanofluid on the weight loss rate was much higher with a shorter period ($t_0 – t_m$), which defines the time required to reach RWL_{max}. The dependence relation is described by equation (9) with high statistical certainty ($R^2 = 0.945$, $p = 0.054$).

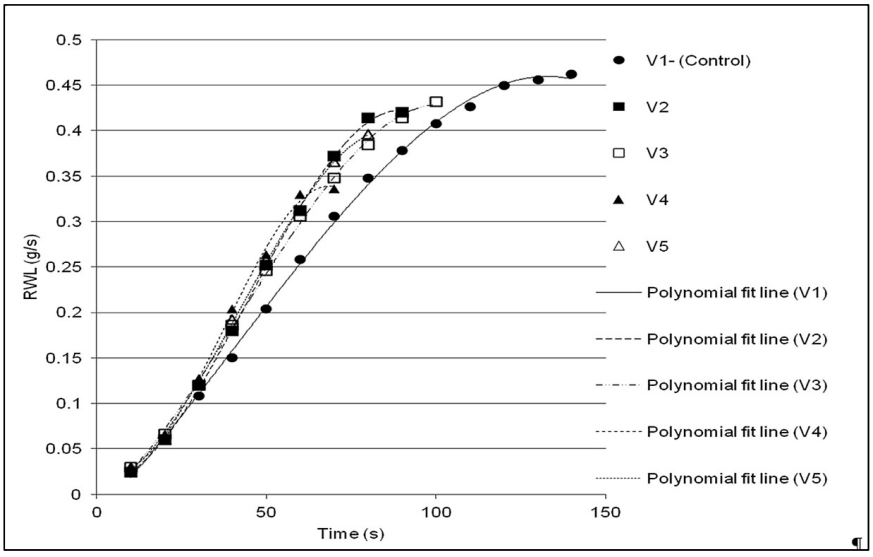

Figure 1. The diagram of the weight loss rate in experimental variants on the time frame $t_0 – t_m$, corresponding to RWL_{max}

$$RWL_{med} = 0.0747x^2 - 0.0821x + 0.1326 \quad (8)$$

$$RWL_{max} = 0.3553x^2 - 0.4165x + 0.4569 \quad (9)$$

where: RWL_{avg} = medium weight loss rate; RWL_{max} = maximum weight loss rate; x – magnetic nanofluid concentration.

The different contribution of magnetic nanofluid to the weight loss rate (RWL) values was also a result of the values of the respective coefficients, equations (8) and (9) describe the variation of this parameter in relation to magnetic nanofluid (MNF), RWL = f(x), where x is represented by MNF). The two equations show higher values of the nanomagnetic fluid coefficients in the equation that describes RWL_{max}, which supports the higher contribution of magnetic liquid to the realization of this parameter.

Conclusions

Biocompatible magnetic nanofluids of the type MF / H_2O (OA-OA) with saturation magnetization 32Gs and density $\rho_{25^oC} = 1.0201$ g/cm^3 influenced some parameters of the controlled drying of wheat seedlings; total drying time, the time required for reaching the maximum weight loss rate, average weight loss rate and the maximum weight loss rate. As an influence of the magnetic nanofluid seed treatment, plant drying duration was increased, with times between 2.15 and 2.35 minutes (9.01 – 24.20%) when compared to the control variant. The drying time required for reaching the maximum weight loss rate was reduced in the variants treated with magnetic nanofluid, with values ranging from 0.40 to 1.10 minute, which represents between 50 and 63.64% as compared to the control variant. The average weight loss rate calculated for the entire drying period as well as the maximum weight loss rate had lower values in the variants treated with magnetic liquid than in the control variant. The results of this research are of interest for managing plant resistance to water stress by alternative methods, based on nanotechnologies that use biocompatible magnetic nanofluids.

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