

## THE CONTRIBUTION OF MAGNETIC FLUID TO THE VARIATION OF THE CHLOROPHYLL CONTENT IN WHEAT

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### ABSTRACT

The present research focused on the contribution of water-based magnetic fluid to the variation of the chlorophyll content in wheat by application associated with urea. Urea and magnetic fluid in various concentrations determined variations of the chlorophyll content in wheat plants, within the limits of  $52.73 \pm 0.15$  -  $57.10 \pm 0.12$  SPAD units, 48 hours after the treatment ( $R^2 = 0.955$ ,  $p < 0.01$ ) and  $52.87 \pm 0.43$  -  $55.53 \pm 0.57$  SPAD units, 10 days after the treatment ( $R^2 = 0.994$ ,  $p < 0.01$ ), respectively. The increase in the chlorophyll content caused by the two factors is variable, and urea has a faster effect than the magnetic fluid. The mathematical equations describing the change in chlorophyll have a high degree of safety. Multivariate analysis facilitated cluster grouping of the variants depending on the results generated, with obvious separation of the variants treated with magnetic fluid. The value of the cophenetic coefficient is 0.998, which provides high certainty to the orientation and cluster grouping of the variants.

**Keywords:** urea, magnetic fluid, chlorophyll, wheat

### INTRODUCTION

The interest in the implications of magnetic fluids in the vegetal field has materialized in various studies. LOBREAUX ET AL. (1992) studied the influence of magnetic nanoparticles coated in perchloric acid on the growth of *Zea mays* plants in the early stages of development. They found that treatment with iron-based magnetic fluid induced changes in the accumulation of ferritin proteins in the roots and leaves of maize plantlets, for three days. SALA (1999) studied the influence of magnetic fluids on some metabolic processes such as seed germination, plant growth and development in the early vegetation stages.

CORNEANU ET AL. (1998) highlighted the stimulating effect of magnetic fluid on the accumulation of starch in the vegetal cell, and GODEANU ET AL. (1998) proved stimulating effects of magnetic fluids on plant growth. Running such a study on species *Mammillaria duwei* on growth medium treated with (water-based and petroleum-based) magnetic fluids, the authors found intensification of the metabolic activity in live tissues. MIHAELA RĂCUCIU and DORINA EMILIA-CREANGĂ (2007) showed the contribution of magnetic fluid to the revitalization of ageing tissue, to the decrease of necrosis and to acceleration of plantlet emergence.

PINTILIE ET AL. (2006) as well as MIHAELA RĂCUCIU, DORINA-EMILIA CREANGĂ (2007) studied the influence of magnetic fluids on vegetal pigments with an assimilating role, especially on the chlorophyll content (a, b) and carotenoids in maize leaves, given that iron is involved in the synthesis of chlorophyll.

In addition, certain phytotoxic effects of different types of magnetic nanoparticles on plant germination and growth in the early vegetation stages of *Cucurbita pepo* (STAMPOULIS ET AL. 2009) and *Cucumis sativus* (PENG ZHANG ET AL. 2012) were discovered.

Starting from the proven effect of ferrofluids on plants, a number of studies investigated the pervasion of magnetic nanoparticles into the vegetal organism, as well as their translocation and circulation to different vegetal tissues and structures (GONZALEZ ET AL. 2008, ZUNY ET AL. 2010, CORREDOR ET AL. 2010).

Recent research in the field of nanotechnologies has focused on the control of chemical substances that protect plants, nutrients respectively, by using magnetic nanoparticles (REMYA NAIR ET AL. 2010).

The present research deals with the contribution of water-based magnetic fluid to the variation in the chlorophyll content in wheat, by applying it in variable concentrations together with urea.

## MATERIAL AND METHOD

The biological material was species *Triticum aestivum* ssp. *vulgare*, Alex cultivar. The crop presented high uniformity regarding plant density and the nutrition and vegetation state. The fertilizer used for foliar application, chosen for its fast effect on plants, was urea, in a concentration of 10% in aqueous solution. The magnetic fluid used was one based on

### MF

water, biocompatible  $\text{H}_2\text{O}$ , with saturation magnetization. The magnetic fluid was used in concentrations of 0.1%, 0.5% and 1% in urea solution. The variants were as follows:  $V_1$  (Mt),  $V_2$  – Urea 10%,  $V_3$  – Urea10% + MF0.1%,  $V_4$  – Urea 10% + MF0.5%,  $V_5$  – Urea 10% + MF1%. The solutions were applied uniformly with a carried sprayer, during stem elongation (BBCH 35-37, flag leaf just visible). The determinations were made 48 hours and 10 days after the treatment. The indicator the study focused on was chlorophyll, a photosynthetic parameter in functioning of which both iron and nitrogen (nutrients supplied by the application of treatments) are involved. For this determination, SPAD 502 Plus (Konica Minolta Sensing Inc. Japan) was used.

Processing of experimental data was performed with the statistic module of application EXCEL of Office 2007 and with the programme called Past. Determinations of descriptive statistical analysis were made, as well as correlations, regressions and multivariate analysis.

## RESULTS AND DISCUSSION

The urea and magnetic fluid in different concentrations determined variations in the chlorophyll content of wheat plants within the limits of  $52.73 \pm 0.15$  -  $57.10 \pm 0.12$  SPAD units 48 hours after the treatment, and  $52.87 \pm 0.43$  -  $55.53 \pm 0.57$  SPAD units 10 days after the treatment (*Table 1*). The distribution of the chlorophyll content based on the complex action of the two factors 48 hours after treatment application can be described by relation (1) in conditions of high statistical certainty ( $R^2 = 0.955$ ;  $p < 0.01$ ) (*Figure 1*).

$$Y = -0.5632x^2 + 4.1918x + 49.112 \quad (1)$$

where:  $x = U + LM$ , representing the cumulative effect of the two factors

The individual contribution of the two factors in the chlorophyll content recorded 48 hours after the treatment is described by relation (2) in conditions of statistical certainty ( $R^2 = 0.828$ ,  $p = 0.171$ ).

$$Chl_{2\text{ days}} = 52.71 + 0.3348U + 0.2621 LM \quad (2)$$

where:  $U = \text{urea}$  ,  $LM = \text{magnetic liquid}$

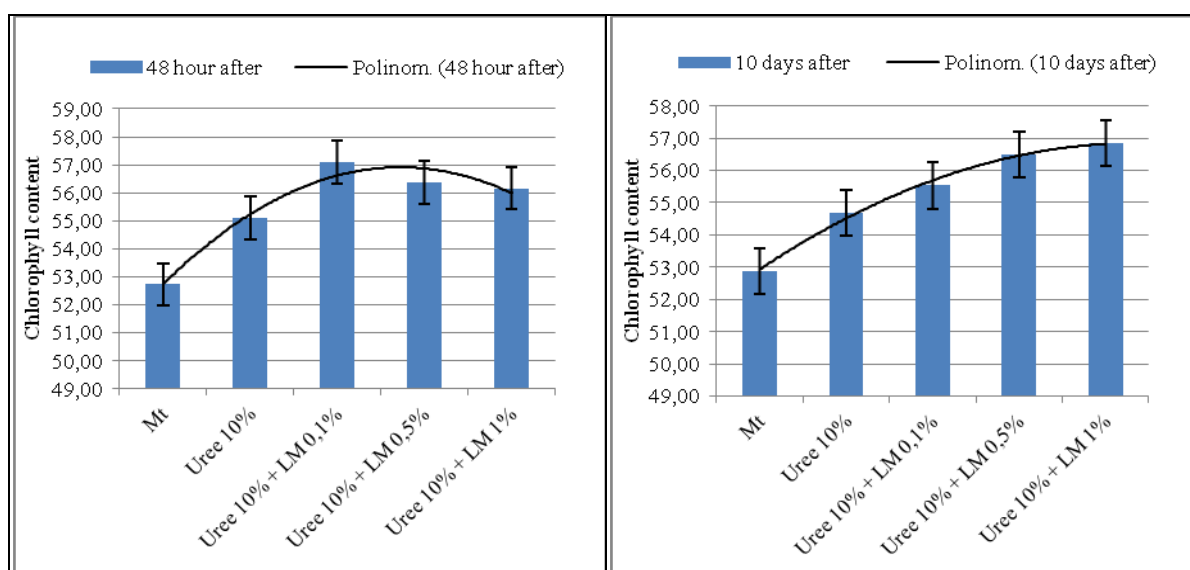
**Table 1. The variation of the chlorophyll content in wheat plants under the influence of urea and magnetic fluid**

Experimental variant	Chlorophyll	Mean chlorophyll content (SPAD units)		
		Before the treatment	48 hours after	10 days after
Mt	52.71±0.16		52.73±0.15	52.87±0.43
Urea 10%			55.11±0.11	54.70±0.55
Urea 10% + LMA 0.1%			57.10±0.12	55.53±0.57
Urea 10% + LMA 0.5%			56.37±0.18	56.49±0.58
Urea 10% + LMA 1%			56.16±0.14	56.83±0.41

The distribution of chlorophyll content 10 days after treatment application, based on the complex action of the two factors – urea and magnetic fluid, is described by relation (3) with high statistical certainty ( $R^2 = 0.994$ ;  $p < 0.01$ ) (Figure 2).

$$y = -0.2029x^2 + 2.1896x + 50.948 \quad (3)$$

where:  $x = U + LM$  representing the cumulative effect of the two factors



**Figure 1. The distribution of the chlorophyll content in wheat plants 48 hours after treatment**

**Figure 2. The distribution of the chlorophyll content in wheat plants 10 days after treatment**

The individual contribution of the two factors to the chlorophyll content recorded 10 days after the treatment is described by relation (4), also with high degree of statistical certainty ( $R^2 = 0.957$ ,  $p = 0.04$ ).

$$Chl_{10\text{ days}} = 52.87 + 0.2237U + 1.9530LM \quad (4)$$

where:  $U = \text{urea}$  ,  $LM = \text{magnetic liquid}$

The comparative analysis of the experimental data shows that urea has a faster effect

towards the modification of the chlorophyll content in wheat leaves, while magnetic fluid has a slower influence, its potential being seen after a longer period of time and identified 10 days after treatment. This is proven by the values of the coefficients of the two factors (urea and magnetic liquid) in equations (2) and (4), as well as by the actual increase of the chlorophyll content. In the equation that describes the variation of the chlorophyll content in wheat leaves 48 hours after (2), the involvement coefficient of urea is 0.3348, while that of magnetic fluid is 0.2621. In equation (4), which describes the distribution of the chlorophyll content 10 days after treatment, the values of the coefficients for the two factors analysed are 0.2237 for urea and 1.9530 for magnetic fluid.

The importance of magnetic fluid in the increasing variation of the chlorophyll content can be accounted for by iron, in the form of the magnetite present in the structure of the ferrofluid, which enters into plant metabolism after being absorbed at foliar level. Iron is a precursor of chlorophyll and it plays an important part in nitrogen metabolism. MARSCHNER (1995) estimated that nitrogen (N) is one of the most important inorganic nutrients in plants because it is a major constituent of proteins, nucleotides, as well as chlorophyll and numerous other metabolites and cellular components. According to the same author, among the factors which may limit  $\text{NO}_3^-$  assimilation, iron (Fe) plays a crucial role, being a metal cofactor of enzymes of the reductive assimilatory pathway nitrate reductase (NR), nitrite reductase (NiR) and glutamate synthase (GOGAT), all requiring Fe as Fe-heme group or Fe-S cluster. ANDREA BORLOTTI ET AL. (2012) communicate that Fe deficiency has a differential effect on N metabolism in roots and leaves, with particular adaptive mechanisms to nutritional constraint acting at the whole plant level.

**Table 2. Variation of the chlorophyll increase depending on the determination factors**

Parameters Variant	Urea (%)	LMA (%)	Before treatment	After 2 days					After 10 days				
				Chl	Total increase	Increase given by urea	Increase given by LM	$\Delta\text{Chl}_{\text{LM}}$	Chl	Total increase	Increase given by urea	Increase given by LM	$\Delta\text{Chl}_{\text{LM}}$
Mt	-	-	52.71	52.73	-	-	-	-	52.87	-	-	-	-
Urea 10%	10	0	52.71	55.11	2.38	2.38	0	0	54.70	1.83	1.83	0.00	0
Urea 10% + LMA 0.1%	10	0.1	52.71	57.10	4.37	2.38	1.99	1.99	55.53	2.66	1.83	0.83	0.83
Urea 10% + LMA 0.5%	10	0.5	52.71	56.37	3.64	2.38	1.26	0.73	56.49	3.62	1.83	1.79	0.96
Urea 10% + LMA 1%	10	1	52.71	56.16	3.43	2.38	1.05	0.21	56.83	3.96	1.83	2.14	0.34

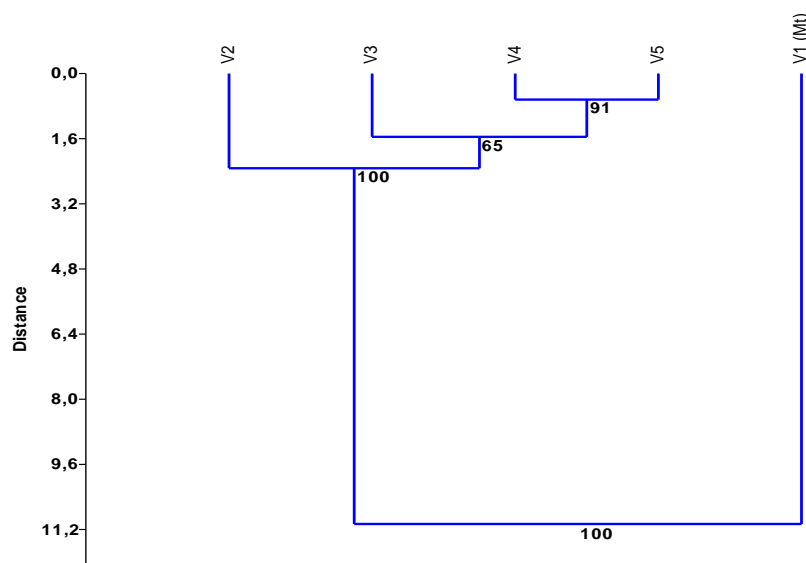
48 hours after treatment application, the variation of the chlorophyll content was made with 2.38 – 4.37 SPAD units, with differentiated contribution of the two factors. Urea determined chlorophyll variation by 2.38 SPAD units, while magnetic fluid determined chlorophyll variation with values between 1.05 and 1.99 SPAD units, as shown in *Table 2*. In the same experimental conditions, in the control variant the chlorophyll content was 52.73 SPAD units, which served as reference for calculating the variation unit.

10 days after treatment application, the total variation of the chlorophyll content was made with 1.83 to 3.96 SPAD units, with differentiated contribution of the two factors. Urea determined an average general chlorophyll increase of 1.83 SPAD units, and magnetic fluid determined chlorophyll increase between 0.83 and 2.14 SPAD units, values that

increase together with the concentration of magnetic fluid (Table 2).

The gradient of chlorophyll increase given by magnetic fluid  $\Delta\text{Chl}_{\text{LM}}$  is on the decrease both 48 hours and 10 days after the treatment.

Multivariate analysis of the experimental results resulted in grouping the variants in two distinct clusters, depending on variant affinities in generating the result. One cluster includes only the control variant ( $V_1$ ), and the second one includes the four treated variants, subgrouped into two clusters. Variant  $V_2$ , where only urea was applied, is positioned separately, while the other three variants ( $V_3 - V_5$ ), where magnetic fluid was also applied, in concentrations of 0.01 – 1% are grouped together, as shown in Figure 3. The value of the cophenetic coefficient is 0.998, which gives high certainty to orienting and grouping the results based on the experimental data analysed.



**Figure 3. Cluster grouping of the variants based on Euclidean distances in relation to the results obtained**

## CONCLUSIONS

Associating water-based magnetic fluid with urea for extra-radicular application is possible, with effects on the upward variation of the chlorophyll content in wheat plants. The singular influence of the two products was quantified differently, it being faster in the case of urea and slower in the case of magnetic fluid. Using the two products together generates a synergetic effect on the variation of the chlorophyll content.

Magnetic fluid can be used for the control and directing of chlorophyll content in wheat by association with urea, and it enhances its effect.

## ACKNOWLEDGEMENTS

The authors thank the Centre for Fundamental and Advanced Technical Research of the Romanian Academy, the Institute for Complex Fluids at the “Politehnica” University from Timișoara, and Dr. Ladislau Vekas, Senior Researcher, for supporting our research by providing the magnetic fluid. The biological material (wheat seeds, Alex cultivar) was provided by the Agricultural Research and Development Station, Lovrin, Romania.

## REFERENCES

- BORLOTTI ANDREA, VIGANI G., ZOCCHI G. (2012): Iron deficiency affects nitrogen metabolism in cucumber (*Cucumis sativus* L.) plants. BMC Plant Biology, 12, p.189 – 204.
- CORNEANU G.C., CORNEANU M., MARINESCU G., BADEA E., BĂBEANU C, BICA D., COJOCARU L. (1998): Effects of magnetic fluids on *Nigella Damascena* (*Ranunculaceae*) under conditions similar to extraterrestrial environment, Abstracts of VIII-th Int. Conf. Magn. Fluids, Timișoara, Romania, p. 447.
- CORREDORE., MARIA C. RISUEÑO, PILAR S. TESTILLANO (2010): Carbon-iron magnetic nanoparticles for agronomic use in plants, Plant Signaling & Behavior, DOI: 10.4161/psb.5.10.13080, p. 1295 – 1297.
- GODEANU A, GODEANU M., STANCA D., CIOBANU I. (1998): The effect of the magnetic fluids on the assimilative pigments biosynthesis of *Spirulina Platensis* (Nordst) Geitl, Abstracts of VIII-th Int. Conf. Magn. Fluids, Timisoara (Romania), p. 451.
- GONZÁLEZ-MELENDE P., R. FERNÁNDEZ-PACHECO, M. J. CORONADO, E. CORREDOR, P. S. TESTILLANO, M. C. RISUEÑO, C. MARQUINA, M. R. IBARRA, D. RUBIALES, A. PÉREZ-DE-LUQUE (2008): Nanoparticles as Smart Treatment-delivery Systems in Plants: Assessment of Different Techniques of Microscopy for their Visualization in Plant Tissues, Annals of Botany 101: 187–195, doi:10.1093/aob/mcm283.
- LOBREAUX S., O. MASSENET, J.F. BRIAT (1992): Iron induces ferritin synthesis in maize plantlets, Plant Mol Biol. 19(4), 563-575.
- MARSCHNER H. (1995): Mineral nutrition of higher plants, London: Academic Press Ltd, Second Edition, p. 232-233, 314-319.
- RĂCUCIU MIHAELA, DORINA-EMILIA CREANGA (2007): Influence of water-based ferrofluid upon chlorophylls in cereals, Journal of Magnetism and Magnetic Materials 311, 291–294.
- RĂCUCIU MIHAELA, DORINA-EMILIA CREANGĂ (2007): Biocompatible Magnetic Fluid Nanoparticles Internalized In Vegetal Tissue, December 15, p. 115-124.
- PINTILIE M., L.OPRICA, M. SURLEAC, C. DRAGUTIVAN, D.E. CREANGA, V. ARTENIE (2006): Enzyme activity in plants treated with magnetic liquid, Rom. Journ. Phys., Vol. 51, Nos. 1–2, p. 239–244, Bucharest.
- REMYA NAIR, SAINO HANNA VARGHESE, BAIJU G. NAIR, T. MAEKAWA, Y. YOSHIDA, D. SAKHTI KUMAR (2010): Nanoparticulate material delivery to plants, Plant Science, Volume 179, Issue 3, p. 154–163.
- SALA F. (1999): Magnetic Fluids Effect upon Growth Processes in Plants, Journal of Magnetism and Magnetic Materials, 201, Ed. Elsevier, North-Holland, p. 440-442. ISSN: 0304-8853.
- STAMPOULIS D., SINHA S.K., WHITE J.C. (2009): Assay-dependent phytotoxicity of nanoparticles to plants, Environ Sci Technol., <http://www.ncbi.nlm.nih.gov/pubmed/19924897> 43(24):9473-9. doi: 10.1021/es901695c.
- ZUNY, C., LAURA CUSTARDOY, J.M. DE LA FUENTE, CLARA MARQUINA, M. R. IBARRA, D. RUBIALES, A. PÉREZ-DE-LUQUE (2010): Absorption and translocation to the aerial part of magnetic carbon-coated nanoparticles through the root of different crop plants, Journal of Nanobiotechnology, doi:10.1186/1477-3155-8-26.
- ZHANG PENG, YUHUI MA, ZHIYONG ZHANG, XIAO HE, ZHI GUO, RENZHONG TAI, YAYUN DING, YULIANG ZHAO, ZHIFANG CHAI (2012): Comparative toxicity of nanoparticulate/bulk Yb<sub>2</sub>O<sub>3</sub> and YbCl<sub>3</sub> to cucumber (*Cucumis sativus*), Environ. Sci. Technol., 46 (3), p. 1834–1841, DOI: 10.1021/es2027295.