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EVALUATION OF THE EFFICIENCY OF FERTILIZERS BY USING THE LABELLED NITROGEN

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Abstract

It is known that extraradicular fertilizers or those containing substances that have a growth-stimulating effect indicated that only the use of biostimulants in the treatment of crops does not often lead to significant effects on production and quality. In these cases, the "explosive" vegetative development of the plant must be supported by an additional nutrients contribution necessary for nutrition. This phenomenon is obvious in the case of poor basic fertilization, on degraded soils, as well as in the case of unbalanced fertilization in macronutrients. The carried out research aimed to establish, using as a 15N isotope tracer, the contribution of foliar fertilizers containing natural organic substances, to increase the efficiency of the use of different forms of nitrogen from the soil applied fertilizer.

The efficient use of nitrogen has a positive effect on the quality of the sunflower crop. The incorporation into the NPK matrix with protein hydrolysate, substances that also have a role of biostimulator has led to increases in the production of green mass, up to 6% compared to the control fertilizer.

***Key words**: fertilizer, sunflower, protein hydrolysate, isotope, labelled nitrogen.*

INTRODUCTION

Agroecosystems rely on inputs including organic and chemical fertilizers with nitrogen to support high productivity. Many fertilizers are composed of organic substances that contain, in addition to other elements, different amounts of organic nitrogen, such as, protein hydrolysate (Colla et al., 2014; Sestili et al., 2018), humic substances (Olivares et al., 2017; Tudor et al., 2017), plant or algae extracts (Craigie et al., 2011; Möller et al., 2015; Amirkhani et al., 2016). However, nitrogen is an extremely mobile element, which could make efficient management a difficult task regarding situations where significant losses can occur due to leaching such as nitrate, ammonia leakage and nitrogen gas emissions (Zhang et al., 2012; Inselsbacher et al., 2013).

Generally, the products involving biostimulant role are organic substances, which, applied in low concentrations, take part in the physiological processes of plant growth and development, with favorable effects, both quantitative and qualitative on the crops, while reducing the losses due to the transport and storage of the products, reducing the pollutant impact of chemical fertilization on the environment at the same time (Yang et al., 2013; Colla et al., 2014; Tudor et al., 2017).

There is a tendency for nitrogen overfertilization to increase production in sunflower crops but it has been observed that the yield can be about 50% (Scheiner et al., 2002).

On this line, remarkable results have been achieved during the investigation of the nutrients sources, their speed circulation in plants and interaction between them, as factors of particular importance in examining the efficiency of the imputations of nutrients in the culture system (Bengtsson & Bergwall, 2000; Takebayashi et al., 2010).

The use of nuclear techniques allows acquaintance providing great accuracy the quantities of nutrients differentially absorbed by plants from soil and from fertilizers following their evolution in plants, as well as the transformations that take place in the soil-plant-fertilizer complex system (Reddy et al., 1993; Scheiner et al., 2002; Stevens et al., 2005;

Watzka & Buchgraber, 2006; Rasmussen et al., 2008). Also, in the researches carried out using the 15N isotope on sunflower plant, the mechanisms of penetration and translocation in the different organs of the plants, of the three forms of existing nitrogen in fertilizers, amidic, nitric, ammonia have been followed.

MATERIALS AND METHODS

A scientific approach regarding rational use of fertilizers, as well as the analysis of nutritional processes can be addressed for application on soil and plant, and it can be done using stable and/or radioactive isotopes (Mihalache et al., 2019).

The paper presents the results obtained from the experiments that took place in the vegetation house on the sunflower plant. The aim was to evaluate the effect of foliar application of some organic fertilizers, containing protein hydrolysate of plant origin and various forms of mineral nitrogen marked 15N.

The two fertilizers were prepared in the laboratory:

- FERT fertilizer including an NPK matrix containing secondary elements and microelements and
- HIDROFERT including NPK type matrix containing secondary elements, microelements and hydrolyzed soy protein.

The vegetable protein hydrolysate used for introduction into the NPK-type fertilizer matrix was acquired from soybean grist with a nitrogen content ranging between 7 and 8%, following the mixed hydrolysis with a first chemical and subsequent enzymatic step with Alcalase 2.4 L, for 3 hours under 65^0C, using a pH of 8, followed by stabilization for 30 minutes under 85^0C. The degree of hydrolysis of sunflower meal ranged between 56 and 60% relative to the total nitrogen composition.

The fertilizers experimentally achieved had the following chemical characteristics, presented in the Table 1.

Table 1. Chemical characteristics of FERT and HIDROFERT fertilizers

Chemical characteristics	**FERT**	**HIDROFERT**
	Content (%)	
Nitrogen, N total, including:	18.52	21.16
ammoniacal	3.62	3.98
nitric	5.41	6.05
amidic	9.49	9.45
organic	0	1.68
Phosphorus, P_2O_5	18.34	19.16
Potassium, K_2O	18.18	18.97
Boron, B	0.01	0.01
Copper, Cu	0.005	0.006
Iron, Fe	0.047	0.052
Magnesium, MgO	0.23	0.25
Manganese, Mn	0.023	0.025
Molybdenum, Mo	0.001	0.001
Sulfur, SO_3	0.45	0.51
Zinc, Zn	0.01	0.01
Organic substances, including:	0.57	10.42
protein hydrolysate	0	9.7
free amino acids	0	0.08
pH	6.58	6.72

The experiments were organized in the vegetation house, having as a test the sunflower (*Helianthus annuus* L.) NEOMA variety. The choice of sunflower as a test plant was due to the advantage offered by the foliage, which has a great holding power of the fertilizer solution when it is applied to the leaves.

The agrochemical experiments on the sunflower culture, the NEOMA variety, were carried out on a chernozem soil with the following physico-chemical characteristics (Table 2).

Table 2. Physico-chemical characteristics of the used soil

Humus	**Nitrogen**	**Mobile phosphorous (P_{AL})**	**Mobile potassium (K_{AL})**	**C_{org}**	**Mobile forms of cations in solution of ammonium acetate + EDTA at pH = 7**				**pH**
					Zn	Cu	Fe	Mn	
(%)	(%)	(mg/kg)	(mg/kg)	(%)	(mg/kg)				pH unit
3.48	0.17	146	224	2.01	13.1	2.74	86	8.6	6.78

The experiences that have taken place have involved the following activities:

- agrochemistry characterization of the used soil;
- organizing and setting up the experience in Mitscherlich vegetation vessels type containing 10 kg cambic loam soil;
- basic fertilization by incorporation into the soil, before sowing ($N_{180}P_{45}K_{45}$), this means 180 kg of nitrogen, 45 kg of phosphorus and 45 kg of potassium per hectare;
- watering responsibilities, at about 50% of the water capacity of the soil;
- the sowing itself, making sure that the seed material is more uniform, calibrated (appearance, weight);
- fertilization using fertilizers containing ^{15}N isotopically labeled nitrogen in the amidic, ammoniacal and nitric groups after sprouting with a dose of 30 mg ^{15}N/pot and 10 mg ^{15}N/plant;
- the maintenance of the plants, following daily watering conditions using 70% water of the field capacity;
- diluted fertilizer solutions preparation and application on plants, 10 ml solution / 1% plant concentration must be established;
- number of foliar treatments 3 following intervals of 7 days.

For each of the combinations of experimental factors, 3 experiments of 3 plants per vessel were provided.

The experimental scheme of agrochemical testing is presented in Table 3.

Table 3. Experimental scheme of agochemically testing

No. var.	Codes	Basic fertilization	Foliar fertilization	^{15}N nitrogen species applied
V1	WBF	Without basic fertilization	Without foliar application	-
V2	WBF+FERT	Without basic fertilization	Foliar application Fert	-
V3	WBF+HFERT	Without basic fertilization	Foliar application Hidrofert	-
V4	BF + 15N-NH2	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	$^{15}N-NH_2$
V5	BF + 15N-NH4	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	$^{15}N-NH_4$
V6	BF + 15N-NO3	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	$^{15}N-NO_3$
V7	BF + 15N-NH2 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Fert	$^{15}N-NH_2$
V8	BF + 15N-NH4 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Fert	$^{15}N-NH_4$
V9	BF + 15N-NO3 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Fert	$^{15}N-NO_3$
V10	BF + 15N-NH2 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Hidrofert	$^{15}N-NH_2$
V11	BF + 15N-NH4 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Hidrofert	$^{15}N-NH_4$
V12	BF + 15N-NO3 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Hidrofert	$^{15}N-NO_3$

The following ^{15}N labeled fertilizers applied by incorporation into soil using a dose of 30 mg / vessel were used in the experiments:

- 20% amide ($-NH_2$) labeled ^{15}N nitrogen urea;
- 20% nitric ($-NO_3$) labeled ^{15}N nitrogen ammonium nitrate;
- 20% ammoniacal ($-NH_4$) labeled ^{15}N nitrogen ammonium nitrate.

After 45 days of sprouting, these plants were harvested as green mass and dried in order to perform isotopic examination and analyze the chemical elements content. (N, P, K, Ca, Mg, Zn, Cu, Fe and Mn).

RESULTS AND DISCUSSIONS

The isotopic determinations of the dried plant material samples were performed using a Thermo Delta V mass spectrometer (IRMS) with an interface for elemental analysis NC 2500 and they were pursued:

- examining the isotopic ratio (R) or the percentage of atoms, AT% $^{15}N/^{14}$, in the samples of plant material depending on the ^{15}N species applied;
- examining the $\delta^{15}N$ (delta) parameter, which represents the accumulation of the ^{15}N isotope in the analyzed sample. This represents the corrected value of the ^{15}N isotope measured against a primary reference scale. The main reference scale for $\delta^{15}N$ used was atmospheric air. The value of $\delta^{15}N$ is given by the formula $\delta^{15}N=1000$ (Rsample-Rstandard)/Rstandard and expressed in units per million (‰);
- ^{15}N isotope export in sunflower plant according to the ^{15}N species applied;
- total nitrogen (%).

The experimentally acquired results and their interpretation are presented in the Figures 1, 2 and 3.

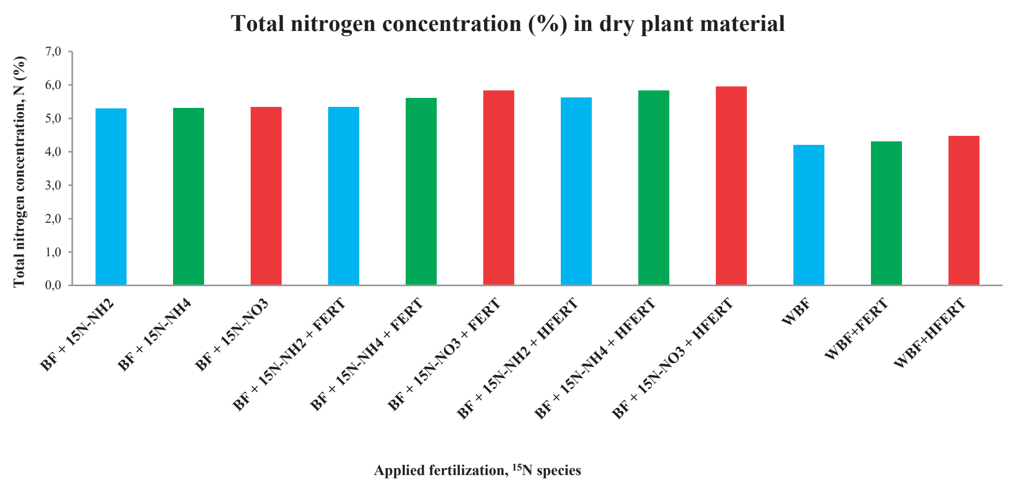

Figure 1. Evolution of nitrogen concentration in plant material depending on the basic and foliar fertilization applied

The nitrogen concentration examined in the vegetal material ranged between 4.21% for the variant without basic or foliar fertilization and 5.93% for the variant using basic fertilization and Hidrofert foliar fertilizer.

Figure 1 shows that the foliar application of the 2 fertilizers on the soil fertilized with NPK fertilizer led to an increase in the concentration of nitrogen in dry plant material for each form of nitrogen.

The highest concentrations of nitrogen accumulated in the plant biomass were recorded for Hidrofert foliar application.

A more accurate picture of the export phenomenon of nitrogen nutrient depending on the applied fertilization can be seen in Figures 2 and 3. In the case of applying foliar fertilizers on a soil without basic fertilization, nitrogen increases (relative values) ranged between 2 and 6%, and in the case of basic fertilization this increase was 32-37%. Basic fertilization led to a 20% increase in nitrogen in the unfertilized control plant, and the increase brought by foliar fertilization on a soil with basic fertilization was 5% for Fert and 9% for Hidrofert with hydrolysed protein fertilizer matrix.

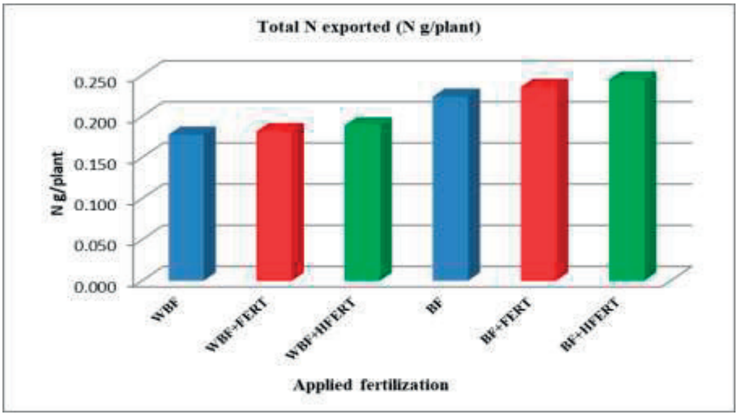

Figure 2. Total nitrogen export depending on the applied fertilization

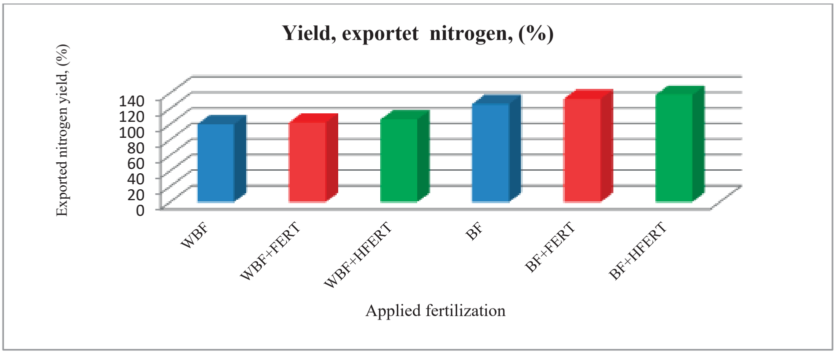

Figure 3. Exported nitrogen yield depending on the applied fertilization

The isotopic determinations of plant material regarding the content and the way of assimilation from the soil into the plant of the nitric, ammoniacal and amidic nitrogen species, have been followed:

- ➢ determination of the isotopic ratio (R) AT% $^{15}N/^{14}N$ in the plant material samples according to the ^{15}N species applied;
- ➢ determining the $\delta^{15}N$ (Δ) parameter, representing the accumulation of the ^{15}N isotope in the analyzed sample (‰);
- ➢ the export of ^{15}N isotope in the sunflower plant according to the ^{15}N species applied radically;

The experimentally acquired results and their interpretation are presented in the following figures (Figures 4 ÷ 7).

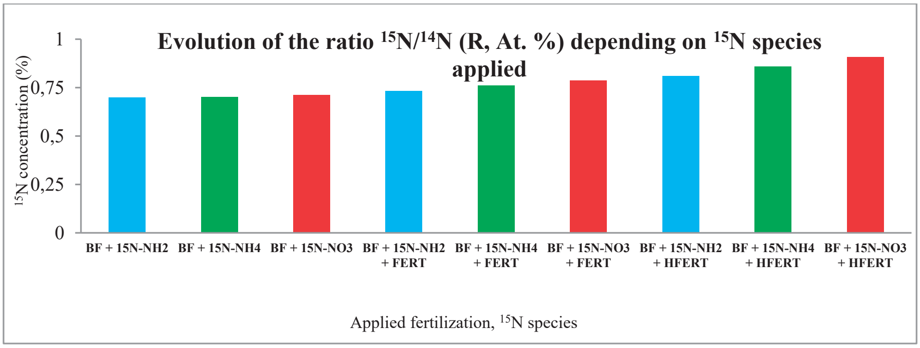

Figure 4. Evolution of the ratio (R, AT $^{15}N/^{14}N$ %) depending on the basic and foliar fertilization applied

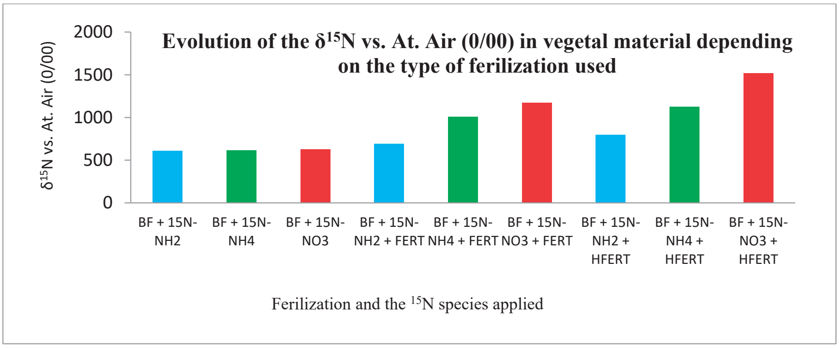

Figure 5. Evolution of the parameter $\delta^{15}N$ (Δ), representing the accumulation of the ^{15}N isotope in the analysed sample according to the species of marked nitrogen radically applied and foliar fertilization

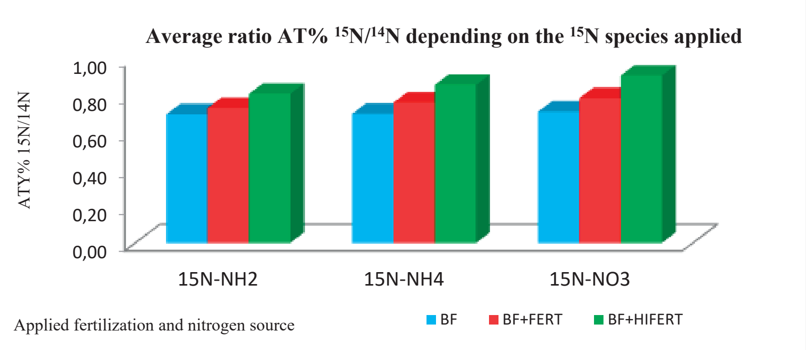

Figure 6. Evolution of the average ratio (R, AT $15N/^{14}N$ %) depending on the ^{15}N applied species

The analysis of the experimental results revealed that the isotopic ratio (R), AT% $^{15}N/^{14}$ in the plant material samples increased in the order of basic fertilization, basic fertilization including Fert fertilizer foliar application and basic fertilization including Hidrofert (HFert) foliar application (Figure 5). The average increase of the R isotopic ratio was 8% compared to only the basic fertilization variant for foliar application of Fert fertilizer and 21.9% for HidroFert (HFert) fertilizer. The foliar application of the HidroFert fertilizer (HFert) has led to an increase of 12.9% for the isotopic ratio compared to the Fert fertilizer variant.

Depending on the nitrogen species applied, an increase in the isotopic ratio (R) was noted as follows: amide nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$), with an increase of 2.1% for the variant using only basic fertilization, of 7.3% in the case of the variant in which Fert foliar fertilization was applied and of 12.2% in the case of the variant in which HidroFert (HFert) foliar fertilization was applied (Figure 6).

For the parameter $\delta^{15}N$ (Δ) (‰), representing the accumulation of the ^{15}N isotope, it increased ascending from basic fertilization, basic fertilization including Fert fertilizer foliar application and basic fertilization including HidroFert (HFert) foliar application. The increase of $\delta^{15}N$ (Δ) was 55.4% compared to only the basic fertilization variant for Fert foliar application and 86.3% for HidroFert (HFert) application. The foliar application of the HidroFert fertilizer (HFert) has led to an increase with 19.9% compared to the Fert fertilizer variant.

Depending on the applied nitrogen species, an increase of the parameter $\delta^{15}N$ (Δ) in the order of amidic nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$) was noted, with an increase of 2.8% in the case variant using basic fertilization alone, 70.1% for the variant in which Fert foliar fertilization was applied and 90.2% for the variant in which HidroFert (HFert) foliar fertilization was applied (Figure 7).

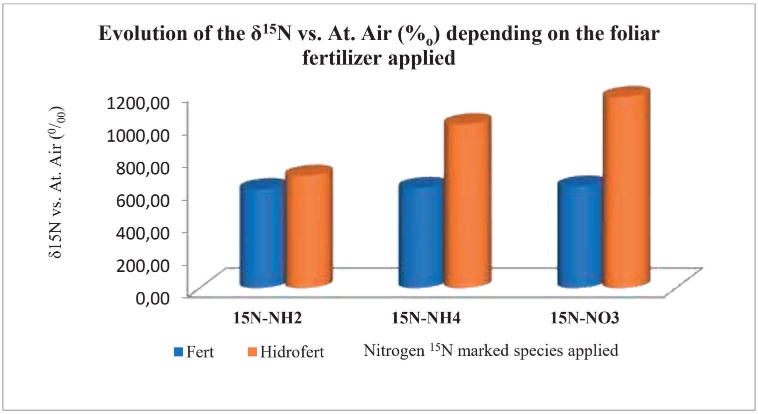

Figure 7. Evolution of the parameter $\delta^{15}N$ (Δ) (‰) depending on the foliar fertilizer used and the nitrogen ^{15}N marked species applied

CONCLUSIONS

In order to evaluate the degree of translocation of different nitrogen forms from the soil into the plant, the nuclear technique, with the ^{15}N labeled isotope applied by incorporation into the soil, was used. The degree of translocation was evaluated using the sunflower test plant (*Helianthus annuus* L.). The procedure was performed under foliar application conditions of two fertilizers containing an NPK matrix including microelements, with / without organic protein hydrolysate.

The isotopic ratio (R), AT% $^{15}N/^{14}N$, in plant material samples increased as follows: basic fertilization, basic fertilization including Fert foliar application and basic fertilization including HidroFert (HFert) foliar application, ranged from 0.698% (15N-NH_2 species) to 0.908% (15N-NO_3 species).

Depending on the nitrogen species applied, an increase in the isotopic ratio (R) as follows: amide nitrogen (-NH_2) < ammoniacal nitrogen (-NH_4) < nitric nitrogen (-NO_3), with an increase of 2.1% in the case of the variant using only basic fertilization, up to 12.2% in the case of the variant in which HidroFert (HFert) foliar fertilization was applied.

Depending on the applied nitrogen species, an evolution of the parameter δ15N (Δ) was noted as follows: amide nitrogen (-NH_2) < ammoniacal nitrogen (-NH_4) < nitric nitrogen (-NO_3), with an increase of 2.8% in the case of the variant using only basic fertilization, of 70.1% in the case of the variant in which Fert foliar fertilization was applied and of 90.2% in the case of the variant in which HidroFert (HFert) foliar fertilization was applied.

The recovery rate for nitrogen due to foliar fertilization alone ranged between 14.75% (species 15N-NH_2) and 26.53% (species 15N-NO_3). The nitrogen recovery rate due to the presence of protein hydrolysate in the fertilizer matrix was not contrasting for the ^{15}N labeled species and ranged between 24.19% (15N-NH_2 species) and 24.89% (15N-NH_4 species).

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