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EVALUATION OF THE EFFICIENCY OF A BIOSTIMULANT CONTAINING ORGANIC SUBSTANCES BY USING LABELED NITROGEN 15N

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Abstract

Fertilizers with foliar application or those containing substances that have a nutrient-stimulating effect have indicated that the use of biostimulants alone in crop treatment often does not lead to significant effects on yield and quality. The carried out research aimed to establish, using the labeled nitrogen 15N as a tracer, the contribution of complex foliar fertilizers containing natural organic substances, to increase the efficiency of using different forms of nitrogen from the soil applied fertilizer. The degree of recovery from soil to plant was evaluated using the sunflower test plant (Helianthus an-nuus). The procedure was performed under foliar application conditions of two fertilizers containing macronutrients, secondary elements and microelements with / without organic substances (protein hydrolyzate). Stable 15N isotopes have been used to examine nitrogen (15N) uptake from soil-applied chemical fertilizers. Depending on the nitrogen species applied, an increase of the 15N/N ratio was observed as follows: amide nitrogen (-NH_2) < ammoniacal nitrogen (-NH_4) < nitric nitrogen (-NO_3).

Key words*: foliar fertilizers, labeled nitrogen 15N, protein hydrolysate, sunflower, biostimulants.*

INTRODUCTION

The use of fertilizer products from the class of biostimulators for agriculture is expanding rapidly (Chiaiese et al., 2018; Xu L. et al., 2018; du Jardin, 2015; Calvo et al., 2014). Applied to plants, seeds or soil, many formulations are available today thanks to intensive research and continuous experimentation that provides information on their effectiveness and mechanisms of action (Caradonia et al., 2019; Garcia-Gonzalez et al., 2016; Michalak et al., 2016; Sharma et al., 2014).

Many fertilizers contain organic substances from the category of protein hydrolysates (Gupta et al., 2021; Gimondo et al, 2019; Maurya et al., 2016; du Jardin, 2015; Shak, et al., 2014), humic substances and fulvic acids (Gupta et al., 2021; Olivares et al., 2017; Tudor et al., 2017; Narwal et al., 2006; Chung et al., 2000), plant or algae extracts (Consentino et al., 2021; Gupta et al., 2021; Hashem et al., 2019; Ronga et al., 2019; Battacharyya et al., 2015), which have the ability to stimulate the metabolism of nutrients and to facilitate absorption of ionic species or molecules (Gupta, S. & Van Staden, 2021; Colla G. et al., 2017; Colla, G. et al., 2014).

In general, products with a bioregulatory role are organic substances, which, applied in small concentrations, participate in the physiological processes of plant growth and development, with favorable effects, both quantitative and qualitative, on crops, contributing to reducing the polluting impact of chemical fertilization on the environment (Bartucca et al., 2022; Del Buono and D. Can, 2021; Wan et al., 2021; Ronga et al., 2019; Salvi et al., 2019; Colla et al., 2017, Tudor et al., 2017; Amirkhani et al., 2016; Colla et al., 2014).

One way to track the efficiency of nitrogen fertilizer uptake by plants is to use nitrogen labeled with a stable isotope such as 15N. Stable isotope-labeled (15N) tracers allow knowing the amounts of nutrients differentially absorbed by plants from soil and fertilizers, as well as the transformations that take place in the complex soil-plant-fertilizer system (Congreves et al., 2021; Langelier et al., 2021; Zhang et al., 2021; Anas et al., 2020).

The production of nitrogen fertilizers is energy intensive (Teske et al., 2022) and large amounts of N-fertilizer are currently intensively supplied by growers every season in the form of nitrate, ammonium or urea (Goñi et al., 2021).

The researches carried out have shown that vegetable protein hydrolyzate is a source whose use in fertilizers with foliar application can lead to a decrease in the amount of nitrogen coming from mineral fertilizers that are obtained with high energy consumption.

MATERIALS AND METHODS

The paper presents the results obtained from experiments carried out in a greenhouse for sunflower crop.

Using the labeled nitrogen 15N for the basic fertilization of the sunflower crop, the effect of the foliar application of two fertilizers on the absorption of different forms of nitrogen from the soil into the plant was investigated.

In the experiment carried out, the FERT fertilizer containing macronutrients, secondary elements and microelements and the HFERT product with the same matrix as FERT to which soy protein hydrolyzate was added were used (Table 1).

The vegetable protein hydrolyzate used for the introduction into the HFERT product was obtained from soybeans, applying mixed hydrolysis with a first chemical step and then an enzymatic one with Alcalase 2.4 L.

The compositional characteristics of the fertilizers used are presented in Table 1.

Table 1. Compositional characteristics of FERT and HIDROFERT fertilizers

Compositional characteristics	FERT	HFERT
	Content (%)	
Nitrogen, N total, including:	18.5	21.2
ammoniacal	3.6	3.9
nitric	5.4	6.1
amidic	9.5	9.5
organic	0	1.7
Phosphorus, P_2O_5	18.3	19.2
Potassium, K_2O	18.2	18.9
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:	0.60	10.4
protein hydrolysate	0	9.7
free amino acids	0	0.08
pH	6.58	6.72

The experiments were organized in the vegetation house of the National Research and Development Institute for Soil Science, Agro-chemistry and Environment Protection-RISSA Bucharest, having as test plant sunflower (*Helianthus annuus*), the variety NEOMA. The agrochemical experiments on the sunflower culture, were carried out on a chernozem soil with the following physico-chemical characteristics: 3.48% humus, 0.17% nitrogen total, 146 mg/kg mobile phosphorous (PAL), 224 mg/kg mobile potassium (KAL), 2.01% organic carbon, and mobile forms of cations in solution at the level: 13.1 mg/kg Zn, 2.74 mg Cu, 86 mg/kg Fe, 8.6 mg/kg Mn and pH 6.78.

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

- organizing and setting up the experience in pots vegetation containing 10 kg cambic chernozem 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;
- the sowing itself, making sure that the seed material is uniform, calibrated (appearance, weight);
- fertilization using products 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;
- preparation of dilute fertilizer solutions and application to plants, in a dose of 10 ml solution with a concentration of 1% / plant;
- application of 3 foliar treatments at an interval of 7 days apart from the previous one.

The experimental scheme of agrochemical testing is presented in Table 2. Each variant was with three replicates.

Table 2. Experimental scheme of agrochemically 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 HFERT	-
V4	BF + ^{15}N-NH_2	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NH_2
V5	BF + ^{15}N-NH_4	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NH_4
V6	BF + ^{15}N-NO_3	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NO_3
V7	BF + ^{15}N-NH_2 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with FERT	^{15}N-NH_2
V8	BF + ^{15}N-NH_4 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with FERT	^{15}N-NH_4
V9	BF + ^{15}N-NO_3 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with FERT	^{15}N-NO_3
V10	BF + ^{15}N-NH_2 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with HFERT	^{15}N-NH_2
V11	BF + ^{15}N-NH_4 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with HFERT	^{15}N-NH_4
V12	BF + ^{15}N-NO_3 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with HFERT	^{15}N-NO_3

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

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

After 45 days of sprouting, these plants were harvested as green mass, dried and ground in order to perform isotopic examination.

RESULTS AND DISCUSSIONS

The analysis of the achieved results by applying the nuclear technique including the use of the ^{15}N stable isotope concerned the nitrogen recovery rate and the evolution of nitrogen export depending on the applied fertilization and the labeled nitrogen species applied in soil, using the same nitrogen dose (source $^{15}NH_4$-ammonium nitrate, ammonium nitrate-$^{15}NO_3$, $^{15}NH_2$-urea).
The direct method on 15N add is the most appropriate to determine the recovery efficiency of N derived from fertilizers.
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.
The following parameters were evaluated to quantify the effect of soil and foliar fertilization on the sunflower crop:

- nitrogen (N, %);
- examining the isotopic ratio or the percentage of atoms, $^{15}N/N$ (%), in the samples of plant material depending on the ^{15}N species applied;
- examining the $\delta^{15}N$ 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 $\delta^{15}N$ represents the $^{15}N/^{14}N$ ratio and expressed in units per million (‰);
- ^{15}N isotope export in sunflower plant according to the ^{15}N species applied and foliar fertilization applied; The uptake of 15N enriched fertilizer added to soil will result in a 15N/14N ratio greater than 0.3663% within the plant, the extent of which is a reflection of uptake of the labelled 15N fertiliser;
- the recovery rate for ^{15}N isotope applied depending on the species of ^{15}N marked nitrogen applied, due only to foliar fertilization.

Part of the obtained results were presented in a previously published article (Nicu et al., 2021).
In order to evaluate the effect of foliar fertilization on nitrogen uptake from chemical fertilizers applied to the soil, the following parameters in the plant material samples were evaluated:

- the isotopic ratio or the percentage of atoms, $^{15}N/N$ (%), in the samples of plant material depending on the ^{15}N species applied;
- determining the $\delta^{15}N$ parameter, representing the accumulation of the ^{15}N isotope in the analyzed sample (‰);
- the export and the recovery rate of ^{15}N isotope in the sunflower plant according to the ^{15}N species applied in soil.

The results obtained through the analysis of the plant material and their interpretation are presented in the following figures (Figures 1-3):

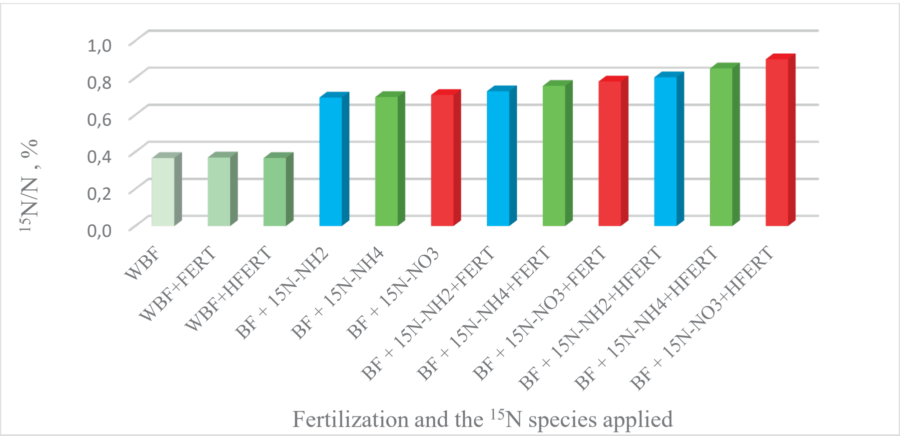

Figure 1. Evolution of the ratio 15N/N, % depending on the basic and foliar fertilization applied

Plants are able to absorb essential elements through their leaves. The absorption takes place through their stomata and also through their epidermis. Base fertilization is essential to ensure adequate nutrition and to make foliar application more efficient (Figure 1). Also, the proteins and amino acids in the HFERT product contributed to a superior uptake and metabolism of the nitrogen nutrient from the basic fertilization, a fact proven with the help of the ^{15}N tracer (Figure 2).

Products containing protein hydrolysates have been shown to be effective with benefits on growth, yield, product quality, resource efficiency and stress tolerance of a wide range of agronomic crops (Rouphael and Colla, 2020; Colla et al., 2017; Calvo et al., 2014).

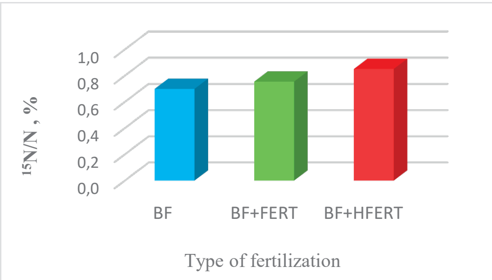

Figure 2. Evolution of the ratio 15N/N, % depending on foliar fertilization

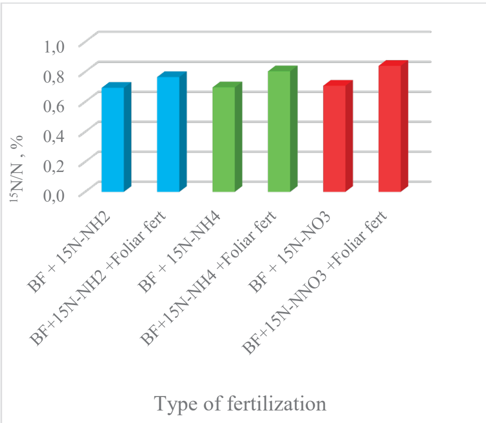

Figure 3. Evolution of the ratio 15N/N, % depending on 15N species applied and fertilization

The analysis of the experimental results revealed that the isotopic ratio $^{15}N/N$ in the plant material samples increased in the order of basic fertilization, basic fertilization including FERT fertilizer foliar application and basic fertilization including HFERT foliar application (Figure 2).

The increase of the $^{15}N/N$ ratio was 7.9% compared to the only basic fertilization variant for foliar application of FERT fertilizer and 21.7% for HFERT fertilizer. The foliar application of the HFERT fertilizer has led to an increase of the $^{15}N/N$ ratio with 12.8% compared to the FERT fertilizer variant (Figure 2). Between the two foliar applied fertilizers the difference was significant by applying HFERT variant.

Compared to the control with the basic fertilization (BF) the differences obtained with the foliar application were statistically significant for FERT and distinctly significant for HFERT (Figure 2).

Depending on the nitrogen species applied, an increase of the $^{15}N/N$ ratio was noted as follows: amide nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$). The application of cumulative foliar fertilization (FERT + HFERT) compared to the control to which no foliar treatments were applied ensured an increase of the 15N/N ratio by 10.3% for ^{15}N-NH_2, by 15.4% for ^{15}N-NH_4 and, respectively, 18.7% in the case of ^{15}N-NO_3 (Figure 3).

The evolution of the isotopic ratio $^{15}N/N$ in the plant material samples and of the parameter $\delta^{15}N$ depending on the used foliar fertilizer and the ^{15}N marked nitrogen species applied in soil, are shown in Figures 4 and 5.

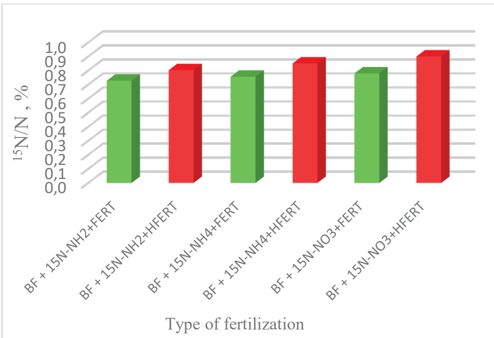

Figure 4. Evolution of the $^{15}N/N$ ratio, %, depending on the foliar fertilizer used and the labeled nitrogen ^{15}N species applied

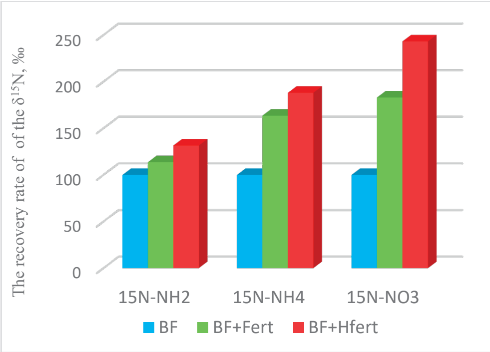

Figure 5. The recovery rate of the $\delta^{15}N$, ‰, depending on the foliar fertilizer used and the labeled nitrogen 15N species applied, compared to the non-foliar fertilized variant (100%)

The compositional difference between the applied foliar fertilizers consists in the fact that the HFERT product contains, in addition to FERT, hydrolyzed soy protein. If we compare the effect of this hydrolyzate on ^{15}N labeled fertilizers applied by incorporation into soil, we notice that the application of the HFERT product leads to increased accumulations in the plant of ^{15}N compared to the FERT product.

Depending on the nitrogen species applied, an increase of the $^{15}N/N$ (%), was noted as follows: amide nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$). The increase due to the variant BF + $^{15}N-NO_3$ + HFERT is 15.4% compared to BF + $^{15}N-NO_3$ + FERT, 12.5% for the $^{15}N-NH_4$ and 10.3% for the $^{15}N-NH_2$ species (Figure 4). These data show us the effect that the protein hydrolyzate has on increasing the root activity of assimilating the nitrogen nutrient from the fertilizers incorporated in the soil, due to the increase in photosynthesis processes.

For the parameter $\delta^{15}N$ (‰), 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 HFERT foliar application. The increase of $\delta^{15}N$ was 55.4% compared to only the basic fertilization variant for FERT foliar application and 86.4% for HFERT application.

The foliar application of the HFERT fertilizer led to an increase for the parameter δ15N (‰), compared to the FERT variant with 15.7% for the $^{15}N-NH_2$ species, 15.0% for the $15N-NH_4$ species and 32.8% for the $^{15}N-NO_3$ species.

Depending on the applied nitrogen species, an increase of the parameter $\delta^{15}N$ in the order of amidic nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$) was noted. By reference to the basic fertilized variant but without foliar application considered 100%, these increases were between 13.6% ($^{15}N-NH_2$) and 83.1% ($^{15}N-NO_3$) for the FERT variant. The same trend is maintained for the variants where the HFERT product was applied, but the increases were between 31.5% ($^{15}N-NH_2$) and 143.2% ($^{15}N-NO_3$) (Figure 5).

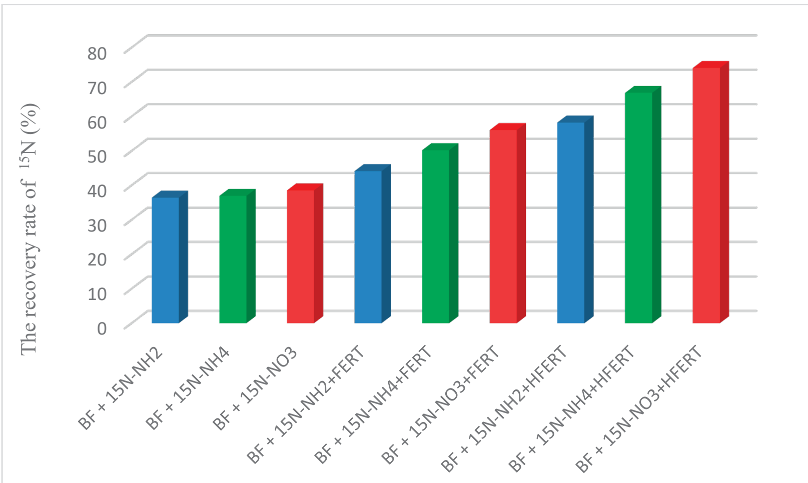

Figure 6. Evolution of the recovery rate for the labeled nitrogen (^{15}N, %) applied depending on the used fertilization and the nitrogen species marked ^{15}N applied

The average recovery rate for isotopically labeled nitrogen ^{15}N (%) depending on the used fertilization was 37.30% for the variant using basic fertilization only, 50.13% in case of additional application of FERT foliar treatment and 66.38% for the application of HFERT foliar treatment (Figure 6).

The data obtained in this study are slightly higher than those presented by other authors (Yan et al., 2020), in which fertilizer ^{15}N recovery for different crops was between 23% and 30%, but these are consistent with the non-foliar fertilized variants (BF + $^{15}N-NH_2$, BF + $^{15}N-NH_4$, BF + $^{15}N-NO_3$) which ranged from about 36% to 38%.

The isotopically labeled nitrogen recovery rate, depending on the applied species, as well as that due only to the foliar application of the two fertilizers, are shown in Figure 7. The highest rate of nitrogen recovery was noted for the nitric form (65.06%), followed by the ammoniacal and the last one for the amidic form (51.19%), regardless of the foliar applied fertilizer.

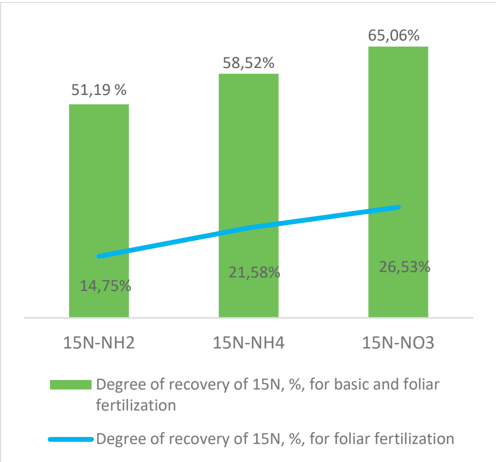

Figure 7. The degree of recovery of ^{15}N for basic and foliar fertilization and due only to foliar fertilization

The assessment of the recovery rate for nitrogen due to foliar fertilization alone ranged between 14.75% ($^{15}N-NH_2$) and 26.53% ($^{15}N-NO_3$) Figure 7. In the case of the corn crop, the application of nitrogen-15 enriched ammonium nitrate showed an efficiency of fertilizer use between 43 to 57% of applied N (Reddy and Reddy, 1993).

The nitrogen recovery rate due to the presence of protein hydrolysate in the fertilizer's matrix was not different for the ^{15}N labeled species and ranged between 24.19% ($^{15}N-NH_2$ species) and 24.89% ($^{15}N-NH_4$ species). These data show us that the positive effect due to the presence of hydrolyzed protein is not influenced by the form of nitrogen in the basic fertilization.

CONCLUSIONS

In order to evaluate the degree of translocation of different nitrogen forms from the soil into the plant, chemical fertilizers 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*). The procedure was performed under foliar application conditions of two fertilizers containing an NPK matrix including microelements, with / without organic substances (protein hydrolyzate).

The isotopic ratio $^{15}N/N$, in plant material samples increased as follows: basic fertilization, basic fertilization including FERT foliar application and basic fertilization including HFERT foliar application, ranged from 0.69% (BF), ranged from 0.75% (BF+FERT), and to 0.85% (BF+HFERT).

The presence of the protein hydrolyzate in the NPK matrix of the biostimulant ensured an increase in the $^{15}N/Nt$ ratio by 21.7% compared to the unfertilized foliar control and by 12.8% respectively compared to the FERT foliar fertilizer.

Depending on the applied nitrogen species, an evolution of the parameter $\delta^{15}N$ was noted as follows: amide nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$).

The presence of the protein hydrolyzate in the NPK matrix of the biostimulant ensured an increase in the $\delta^{15}N$ parameter by 13.6% in the case of amide nitrogen ($-NH_2$), by 63.6% in the case of ammoniacal nitrogen ($-NH_4$) and by 88.1% in the case of nitric nitrogen ($-NO_3$), by applying the HFERT foliar fertilizer.

The assessment of the recovery rate for nitrogen due to foliar fertilization alone ranged between 14.75% ($^{15}N-NH_2$) and 26.53% ($^{15}N-NO_3$).

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