USE OF ISOTOPIC TECHNIQUES TO EVALUATE FOLIAR FERTILIZATION EFFICIENCY

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ABSTRACT

In this study, the foliar fertilization efficiency was assesed by using nuclear techniques and the stable isotope ^{15}N. The objective was to increase nitrogen use efficiency in plants. In addition, the mobility of nitrogen in plants was evaluated.

The environmentlly friendly impact of this type of fertilizer is supported by means of an increase in nutrients uptake from the natural soil supplies, and as a result of foliar fertilization.

The trials were conducted in INCDPAPM-ICPA Bucharest greenhouse, using sunflower as test plant.

To evaluate the mobility of nitrogen in plants, a PK with mezo and microelements (Mg, S, Fe, Cu, Zn, Mn, Co, Mo) foliar fertilizer was enriched with labeled ^{15}N and unlabeled N, using urea and ammonium nitrate as nitrogen sources. Therefore, 6% of the total nitrogen content was represented by the ^{15}N labeled isotope (in all three forms: ammoniacal, $^{15}N\text{-}NH_4$, nitric , $^{15}N\text{-}NO_3$ and amide, $^{15}N\text{-}NH_2$).

The obtained results suggest that nuclear techniques can be successfully used in agrochemical research to deepen plant nutrition and nutrients translocation studies.

INTRODUCTION

In the recent years, nuclear techniques have been used in agronomic research due to their advantages (i.e. sensitivity) as concerns the results related to plant nutrition, fertilizers application, genetic studies, plant breeding and soil development studies, conservation and preservation of agricultural products, monitoring and implementation of environmental procedures [4]. This methods can accurately determine the amounts of nutrients absorbed by plants from soil, respectively, fertilizers, their mobility in plants, as well as the transformations that occur in the soil-plant-fertilizer complex system [5].

Given that nutrient absorption and metabolism by plants are governed by complex microscopic mechanisms that can not be investigated using conventional methods, the chemical substances used in agriculture, namely the chemical fertilizers are labeled using stable and radioactive isotopes (i.e. ^{15}N, ^{13}C, ^{2}H, ^{14}C, ^{35}S, ^{32}P etc.) [1, 2]. The researches carried out using ^{15}N isotope as carried out to assess the are focused on the absorption mechanisms and mobility in plant of the three forms of nitrogen present in the fertilizers (amide, nitric and ammoniacal) [3].

It was reported that ^{15}N is the isotope most widely used in agricultural research. ^{15}N was separated by ^{14}N - ^{15}N isotope exchange method using nitrogen oxide (NO) and nitric acid (HNO_3) at the National Institute for Research and Development of Isotopic and Molecular Technologies Cluj-Napoca (INCDTIM) [8].

^{15}N isotopic labeling techniques can be used to assess the processes and mechanisms related to nitrogen assimilation and translocations, as well as the metabolic processes occurring in plants and their relationships with the environment [6, 7].

The use of nuclear techniques and stable isotope ^{15}N in different experimental conditions can help to elucidate certain agrochemical problems, as follows:

- nitrogen use efficiency in plants as a result of foliar application or soil incorporation of the fertilizer;

- agrochemical efficiency and quantification of the environmentlly friendly effect of the fertilizer (supported by an increase in nutrients uptake from the natural soil supplies and as a result of foliar /soil incorporation fertilization).

MATERIAL AND METHOD

The experimental activities were focused on:

- identifying the (^{15}N) nitrogen labeled sources, used as a tracer in the experiments;
- identifying the isotope labeling methods that use ^{15}N to evaluate nitrogen mobility in plant;
- selecting the foliar fertilizer;
- fertilizer characterization;
- developing the ^{15}N labeled fertilizer (PK matrix labeled with ^{15}N isotope);
- selecting the test plant and the appropriate experimental design;
- selecting the soil;
- soil characterization;
- selecting treatments (fertilizer solution concentration, application method);
- foliar fertilization of the test plant;
- establishing the sampling moment of the plant material and the set of analyses to be carried out;
- harvesting the plant material; sample preparation for biometric, analytical and isotopic determinations;
- chemical and isotopic determinations;
- interpretation of the results.

The experiments were carried out in INCDPAPM-ICPA Bucharest greenhouse, using sunflower as test plant. The experimental designs consisted of single-factor experiments.

To investigate the mobility of nitrogen in plant and the agrochemical efficiency, experiments were performed using a PK matrix with trace elements foliar fertilizer enriched with labeled ^{15}N and unlabeled N (from urea and ammonium nitrate). 6% of the total nitrogen content was represented by the ^{15}N labeled isotope (in all three forms: ammoniacal, nitric and amide).

The isotopic determinations of the dry calatide samples ($\delta\ ^{15}N$ and $^{15}N/^{14}N$ ratio) and the total nitrogen analyses were performed at the Cornell Isotope Laboratory (COIL), Cornell University, Ithaca, USA :

- Determination of the isotopic ratio (R) or the atoms percentage , AT% $^{15}N/^{14}N$, in the plant samples in accordance to the applied (^{15}N) species. R is the ratio of the heavy to light isotope in the sample;
- Determination of $\delta^{15}N$ vs. At Air - the accumulation of ^{15}N isotope in the analyzed sample. This is the corrected value of the delta isotope for ^{15}N, measured against a primary reference scale. The primary reference scale for $\delta^{15}N$ is the Atmospheric Air. $\delta\ ^{15}N$ values are expressed in units per million (‰).cann be calculated by means of the following formula, $\delta^{15}N = 1000 * (Rsample-Rstandard) / Rstandard$);
- The export of ^{15}N isotope to sunflower calatide, as a function of the foliar fertilization with ^{15}N species;
- The degree of ^{15}N isotope translocation represents the mobility of different forms of nitrogen in plant and can be assessed by using ^{15}N isotope-labeled fertilizers.

<u>Working conditions:</u>

- Mitscherlich pots with 20 kg of dry soil.
- Soil type: Chernozem.
- Test plant: sunflower, NEOMA hybrid.

- Nutrient dose to base fertilization was $N_{90}P_{9}0K_{90}$ using NPK complex fertilizer 15:15:15 (6 g/pot).
- Irrigation 50% of the water capacity of the pot.
- Sowing - uniform seed material (appearance, weight).
- Plants maintenance: daily irrigation up to 2/3 of the water capacity of the pots.
- 3 replicates, 2 plants/pot, for each of the combination of the experimental factors (experimental variants).
- In the experiments was used a PK matrix with trace elements foliar fertilizer enriched with urea and ^{15}N isotope-labeled urea at amide group (urea-$^{15}NH_2$), and ammonium nitrate and ^{15}N isotope-labeled ammonium nitrate at ammoniacal or nitric group ammonium nitrate (ammonium nitrate-$^{15}NH_4$; ammonium nitrate-$^{15}NO_3$).
- Treatments: 15 ml solution(1 %)/ plant (30 ml/pot. The application was performed on each leaf using a 1,0 ml syringe.
- Treatments: 4; interval: 7-days; application: in the morning.

RESULTS AND DISCUSSIONS

The chemical characteristics of the PK foliar fertilizer used as the basis for obtaining the variants of foliar fertilizers and ^{15}N isotope-labeled foliar fertilizers are presented in Tables 1- 2.

Table 1

Chemical characteristics of the PK foliar fertilizer

Chemical characteristics	PK matrix
	(g/l)
Nitrogen (N)	-
Phosphorus (P_2O_5)	70
Potassium (K_2O)	60
Boron (B)	0,300
Cobalt (Co)	0,010
Cuprum (Cu)	0,300
Iron (Fe)	0,400
Magnesium (Mg)	0,300
Manganese (Mn)	0,300
Molybdenum (Mo)	0,010
Sulfur (SO_3)	2,00
Zinc (Zn)	0,100

Table 2

The chemical characteristics of ^{15}N isotope-labeled foliar fertilizers

Chemical characteristics	FOLIAR FERTILIZERS VARIANTS		
	PK matrix AMMONIUM NITRATE UREA-$^{15}NH_2$	PK matrix UREA AMMONIUM NITRATE-$^{15}NO_3$	PK m UREA AMMONIUM NITRATE-$^{15}NH_4$
	Determined values	Determined values	Determined values
	(g/l)		
Total nitrogen from the addition, from which:	120,06	120,45	120,45
like ^{15}N	6,75	6,02	6,02
Phosphorus (P_2O_5)	69,60	70,40	69,80
Potassium (K_2O)	58,80	59,40	58,9
Boron (B)	0,310	0,310	0,310
Cobalt (Co)	0,010	0,010	0,010
Cuprum (Cu)	0,290	0,310	0,280
Iron (Fe)	0,380	0,390	0,410

Magnesium (Mg)	0,290	0,310	0,310
Manganese (Mn)	0,310	0,290	0,290
Molybdenum (Mo)	0,010	0,010	0,010
Sulfur (SO_3)	2,26	2,30	2,34
Zinc (Zn)	0,120	0,130	0,120

Note: *the difference between the experimental variants consists in the nature of the applied ^{15}N*

The physicochemical characteristics of the soil (Chernozem) used in the experiments are presented in the table below.

Table 3

Physicochemical characteristics of the soil

Humus	**Nitrogen**	**Phosphorus (P_{AL})**	**Potassium (K_{AL})**	**pH**
(%)		(mg/kg)		
3,26	0,22	188	162	7,48

In Table 4 is presented the experimental diagram used for the study of the mobility of nitrogen in plant. Variant 1 represents the unfertilized control.

Table 4

Experimental diagram used for the study of the mobility of different forms of nitrogen in the plants

Experimental variants codes	**FERTILIZATION VARIANTS**	
V1	**$N_{90}P_{90}K_{90}$**	**-**
V2	**$N_{90}P_{90}K_{90}$**	**PK foliar fertilizer AMMONIUM NITRATE *UREA-$^{15}NH_2$***
V3	**$N_{90}P_{90}K_{90}$**	**PK foliar fertilizer UREA *AMMONIUM NITRATE-$^{15}NH_4$***
V4	**$N_{90}P_{90}K_{90}$**	**PK foliar fertilizer UREA *AMMONIUM NITRATE-$^{15}NO_3$***

The interpretation of the results was performed using Duncan's multiple range test, at a significance level $\alpha = 0,05$ with SPSS 14.0 software. The results present a 95% confidence level with a 5% error margin. Values followed by the same letter do not differ significantly between them.

In the Table 5 shows the values of the isotopic parameters determined in the dried samples of sunflower calatidium depending on the experimental factors, depending on the applied foliar fertilization variants.

Table 5

The values of isotopic parameters determined in sunflower calatidium

FOLIAR FERTILIZING VARIANTS	**$^{15}N/^{14}N$ ratio**	**$\delta^{15}N$**	**Nt export**	**^{15}N export**	**Degree of ^{15}N translocation**
	(AT %)	(‰)	(g)	(mg)	(%)
	(average values)				
PK foliar fertilizer AMMONIUM NITRATE*UREA-$^{15}NH_2$*	1,03971	1876,66	0,155	1.613	42.9
PK foliar fertilizer UREA *AMMONIUM NITRATE-$^{15}NH_4$*	0,97000	1604,25	0.184	1.787	49.5
PK foliar fertilizer UREA *AMMONIUM NITRATE-$^{15}NO_3$*	1,09300	2026,66	0.160	1,753	55.5

Figure 1 graphically illustrates the influence of foliar fertilization with different ^{15}N isotope-labeled foliar fertilizers on the total nitrogen concentration from sunflower calatidium in comparison to the unfertilized control.

Values followed by the same letter (a) does not show a significant difference from one another. The values obtained for the total nitrogen concentration for all experimental variants do not differ significantly between them.

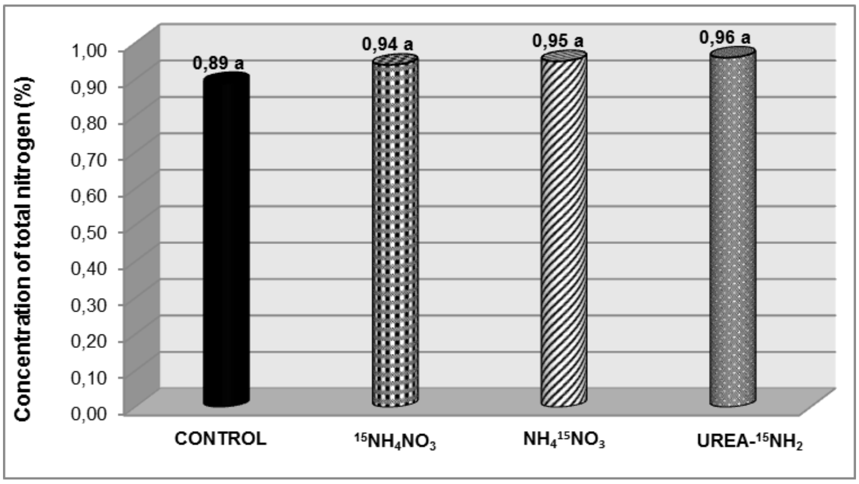

Figure 1. The concentration of total nitrogen in sunflower calatidium according with experimental factors (%)

Figure 2 graphically shows the influence of the fertilization with the different foliar variants that contain with ^{15}N isotope-labeled foliar fertilizers on the ratio $^{15}N/^{14}N$ (%) in sunflower calatidium samples.

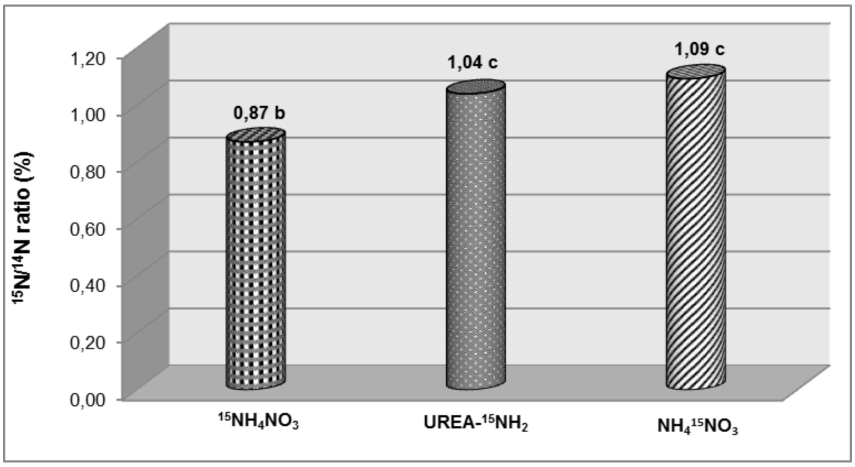

Figure 2. Influence of experimental factors on the evolution of $^{15}N/^{14}N$ ratio in sunflower calatidium (%)

The values of $^{15}N/^{14}N$ ratio obtained for the samples of plant material harvested from variants fertilized with fertilizer containing urea-$^{15}NH_2$ (1,04%) and ammonium nitrate-$^{15}NO_3$ (1,09%) do not present a significant difference from each other, but are significantly higher from the variant that contains ammonium nitrate-$^{15}NH_4$ (0,87%).

Figure 3 graphically shows the influence of fertilization with different foliar variants that also contain ^{15}N isotope-labeled foliar fertilizers (to evaluate the mobility of different forms of nitrogen in plant) on the evolution of δ ^{15}N paramater in sunflower calatidium.

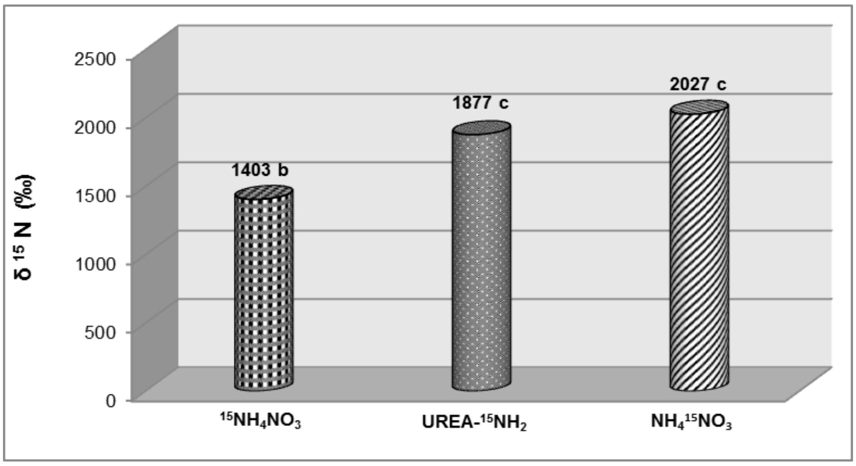

Figure 3. Influence of experimental factors on the evolution of $\delta^{15}N$ parameter in sunflower calatidium (‰)

The values of $\delta^{15}N$ parameter obtained for the samples of plant material harvested from variants fertilized with fertilizer containing urea-$^{15}NH_2$ (1877 ‰) and ammonium nitrate-$^{15}NO_3$ (2027 ‰) do not present a significant difference from each other, but are significantly higher from the variant that contains ammonium nitrate-$^{15}NH_4$ (1403 ‰).

Figure 4 graphically shows the influence of fertilization with the different ^{15}N isotope-labeled foliar fertilizers on the evolution of the total nitrogen export in the sunflower calatidium (g).

The values obtained for the total amount of nitrogen exported from samples of plant material harvested from variants fertilized with foliar fertilizers containing ^{15}N isotopically labeled fertilizer do not differ significantly between them. A higher amount of total nitrogen exported was obtained for variant fertilizing with ammonium nitrate-$^{15}NH_4$ (0,184 g) and the smallest for variants fertilized with foliar fertilizers containing urea-$^{15}NH_2$ (0,155 g).

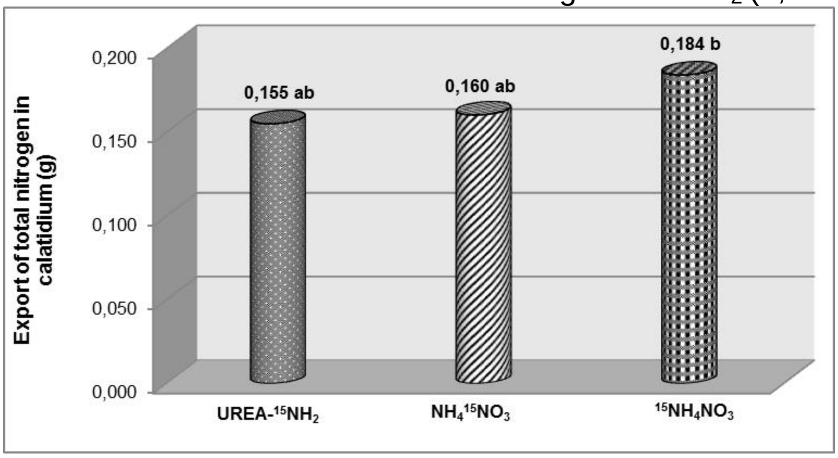

Figure 4. Influence of the experimental factors on the total amount of nitrogen exported in sunflower calatidium (g)

Figure 5 graphically presents the influence of fertilization with the different ^{15}N isotope-labeled foliar fertilizers on ^{15}N export (mg) in sunflower calatidium.

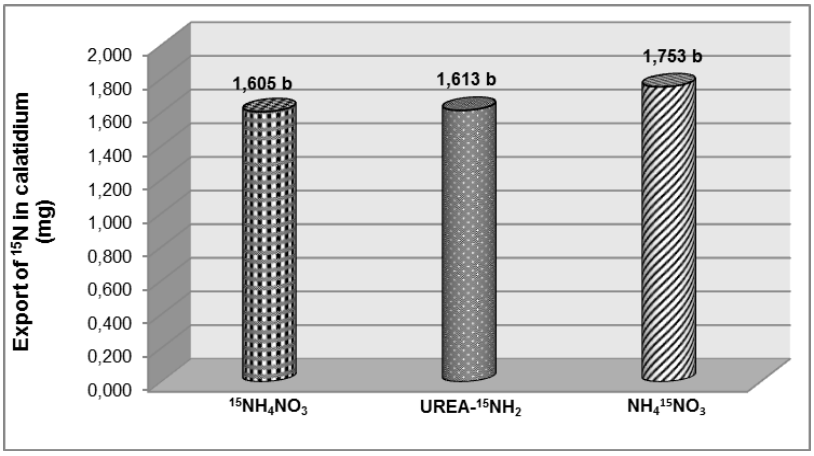

Figure 5. Influence of experimental factors on the amount of ^{15}N exported to sunflower calatidium (mg)

The values for the ^{15}N quantity exported in sunflower calatidium obtained for the samples of plant material harvested from variants fertilized with foliar fertilizers containing ^{15}N isotopically labeled fertilizer do not differ significantly between them.

A higher amount of ^{15}N exported in sunflower calatidium was obtained for the samples collected from variants fertilizing with ammonium nitrate-$^{15}NO_3$ (1,753 mg).

Figure 6 graphically presents the translocation degree of ^{15}N (%) in sunflower calatidium for each foliar fertilizing that contains ^{15}N isotope-labeled fertilizers.

The highest amount of ^{15}N translocated in the sunflower calatidium (48,54 %) was determined in the samples of plant material harvested from variants fertilized with ammonium nitrate-$^{15}NO_3$. The lowest amount of ^{15}N translocated was obtained for plant samples fertilized with urea-$^{15}NH_2$ (39,82 %).

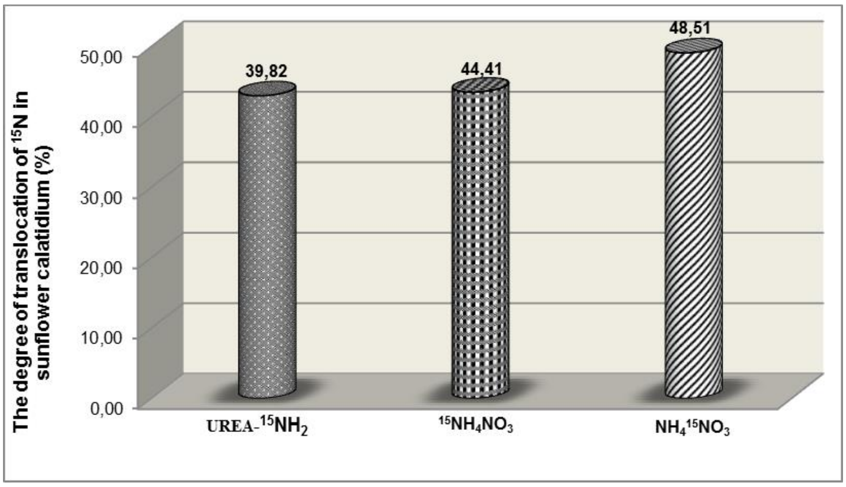

Figura 6. Influence of experimental factors on the translocation degree of ^{15}N in sunflower calatidium (%)

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CONCLUSIONS

- In this study, unlabeled and ^{15}N isotope-labeled (at different nitrogen forms, i.e. amide, nitric and ammoniacal) foliar fertilizers were obtained starting from a PK + trace elements matrix.
- The amount of ^{15}N exported to sunflower calatidium was 1,605 mg for ammonium nitrate-$^{15}NH_4$ and 1,753 mg for ammonium nitrate-$^{15}NO_3$, with no significant differences recorded between the three ^{15}N-labeled nitrogen forms.
- The mobility of nitrogen, expressed as a degree of translocation in sunflower calatide, varied from 39,82% in the case of urea-$^{15}NH_2$ (amide form of nitrogen) to 48,51% in the case of ammonium nitrate -$^{15}NO_3$ (nitric form of nitrogen).
- Using of experimentally obtained foliar fertilizers does not significantly affect the mobility of various forms of nitrogen in the plant.

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