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EVALUATION OF THE EFFECT OF ^{15}N-LABELED FERTILIZERS ON MAIZE PLANT

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Abstract

The effects of the experimental factors studied concerning isotopic indicators ($^{15}N/^{14}N$ isotopic ratio, degree of $\delta^{15}N$ isotopic accumulation, export of ^{15}N isotope, ^{15}N isotope concentration, degree of ^{15}N isotope uptake) were evaluated in maize plant, Cortes hybrid. Experimental foliar fertilizers were obtained: Nutrifert (complex NPK matrix, secondary nutrients and trace elements); Nutrifert vegetal (complex NPK matrix, secondary nutrients, trace elements and vegetal natural organic compounds); Nutrifert Plus (complex NPK matrix, secondary nutrients, trace elements and animal proteins). The natural organic compounds (compounds resulted by neutral hydro lysis of some proteic substances which have biostimulating and chelating action) showed the positive effect of foliar fertilization on nutrients use efficiency by biostimulating nutrients uptake by plant. The experiment was bifactorial with two factors: A, chemical nature of the ^{15}N-labeled nitrogen from the fertilizers applied radicular and B, chemical nature of the foliar fertilizers (with or without vegetal and animal proteic hydrolisates). By combining these two factors, 12 variants were established. The effects induced on isotopic indicators were evaluated. Analytical and isotopic determinations were performed from dried plant material samples. The experimental data were statistically processed by variance analysis using the Duncan multiple comparison test, multiple comparison threshold α=0.05. For the graphic and statistical processing, the Excel, XLSTAT and SPSS 14.0 programs were used.

Experimentally, it has been found that ^{15}N isotope can be used in agrochemical research to improve: the nutrition studies, the research concerning the absorption from soil and mobility in plant of nutrients and of different forms of nitrogen (amide N-NH_2, nitric N-NO_3, ammonical N-NH_4) from nitrogen fertilizers, the experiments for demonstration of the utility of different fertilization methods.

Keywords: plant, ^{15}N isotope, foliar fertilizer, radicular fertilizer, agrochemical testing

1. Introduction

Agrochemistry deals with plant nutrition issues in a close connection with the application of fertilizers, amendments, pesticides and bioregulators. Based on soil-plant-fertilizers relationships, it sets out rules and practical methods for plant nutrition regulation, fertilization and soil fertility evaluation.

In the field of agriculture, it is recommended to use extraradicular fertilizers for: the treatment of certain plant nutrition diseases, preventing the plant diseases, increasing crop yield, product quality and reducing the negative impact of classical fertilizers on the environment. Also, plants treated with fertilizers containing chelating natural organic

compounds are more resistant to frost, to drought, to biotic and abiotic stress (D. MIHALACHE et al., 2015 [1]).

The analysis of the existing literature data on classical extraradicular fertilizers or those with growth-enhancing compounds in their formulas has indicated that only the use of biostimulators in crops treatment, often does not result in some significant effects. In these cases, the „explosive“ vegetative development of the plant is not sustained by an additional, rapid supply of macro and micronutrients (CIOROIANU Tr.M., C. SÎRBU, 2010 [2]).

The widespread use of foliar fertilizers is a current European Community policy which has the aim of reducing the fertilization with classical fertilizers and, mitigating the soil and groundwater pollution, their management following the European environmental protection standards and that's of promoting a sustainable agriculture. In the last years, there have been concerns about the production of new types of foliar complex fertilizers containing natural compounds with biostimulating action. In the same time, it has been highlighted that biostimulators represent a special category of agro-compounds obtained synthetically or naturally, which affect the regulation of the physiological processes in the plant. Generally, bioregulatory products are organic compounds. When they are applied in low concentrations, have positive effects on the physiological processes of plant growth and development and on the quality and quantity of crop yield. (CIOROIANU Tr.M., C. SÎRBU, 2011 [3]).

The nuclear techniques with ^{15}N isotope is useful for some agrochemical aspects related to: the efficient use of the different nitrogen forms (amide, nitric, ammoniacal) from fertilizers containing complex NPK matrix and organic substances with biostimulating and chelating properties (applied foliar and by incorporating in soil) for plants; biostimulating action of nutrients absorbtion from the natural soil resources (D. MIHALACHE et al., 2017 [4]).

For a rigurous evaluation, the processes of nutrients absorbtion and metabolization by plants, determined by complex microscopic mechanisms, were studied by using stable radioactive isotopes (^{15}N, ^{2}H, ^{35}S, ^{13}C, ^{14}C, ^{32}P etc.). (BLAIR G., TILL R., 2000 [5]). ^{15}N is very widely used in the agricultural research. The use of stable ^{15}N in agrochemical testing is very helpful in understanding the nutritional processes of plants, which may lead to productivity and sustainability increases of the agricultural systems.

The use of the stable ^{15}N isotope in agrochemical studies related to evaluation of nitrogen cycle and its transformation from the nitrogen fertilizers is well established, but in case of long term testing (perennial crops), there are some deficiency because of the plants size, sampling procedures, detection levels and of interferences in analytical and isotopic determinations (A. QUIÑONES et al., 2003 [6]; BUSTAMANTE C. et al., 1997 [7]; LIMA F., MALAVOLTA E., 2003 [8]; RAPISARDA P. et al., 2005 [9]).

The objective of the study was to estimate the combined effects of chemical nature of the ^{15}N-labeled nitrogen from the fertilizers applied radicular and of the chemical nature of the foliar fertilizers on the isotopic indicators determined on the maize plant.

Materials and methods

The research was carried out within at the National Research and Development Institute for Soil Science, Agro-chemistry and Environment – ICPA Bucharest, within the Physical-Chemical Analysis Laboratory and the Laboratory of Fertilizer Quality Control and Testing, and the isotopic analyses (determination of $\delta^{15}N$ parameter and $^{15}N/^{14}N$ percentage)

were determined at Cornell Isotope Laboratory (COIL), Cornell University, Ithaca, United States of America. In Table 1 the experimental variants and types of fertilization are presented.

The following experimental foliar fertilizers were obtained: Nutrifert (complex NPK matrix, secondary nutrients and trace elements); Nutrifert vegetal (complex NPK matrix, secondary nutrients, trace elements and vegetal natural organic compounds); Nutrifert Plus (complex NPK matrix, secondary nutrients, trace elements and animal protein). In Figure 1 is presented the technological flow of production of NPK foliar fertilizers used in agrochemical testing.

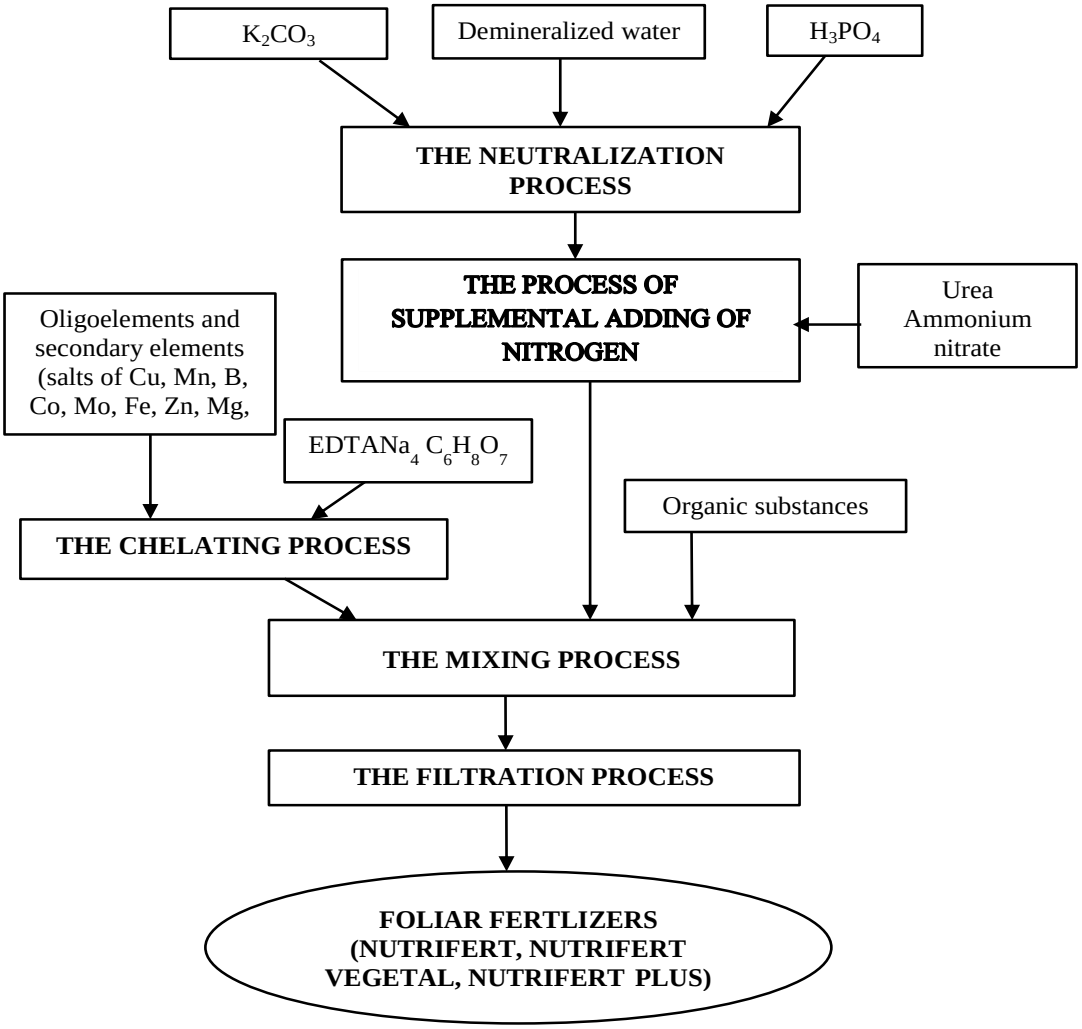

Figure 1. Technological flow of production of NPK liquid fertilizers with secondary nutrients and trace, and organic substances

Within these agrochemical tests, for radicular fertilization, ^{15}N-labeled fertilizers (urea and ammonium nitrate) were used by incorporating in the soil: $(^{15}NH_2)_2CO$, urea with 10% ^{15}N-labeled ($-NH_2$); $^{15}NH_4$-NO_3, ammonium nitrate with 10% ammonia nitrogen ^{15}N-labeled ($-NH_4$); NH_4-$^{15}NO_3$, ammonium nitrate with 10% ammonia nitrogen ^{15}N-labeled ($-NO_3$)

Working conditions:

- Mitscherlich pots with 10 kg of dry soil, type Chernozem.
- Test plant: maize, CORTES hybrid.
- Nutrients dose for basic fertilization was $N_{45}P_{45}K_{45}$ using NPK 15:15:15 (0.8 g/pot), equivalent of 300 kg fertilizer/ha.
- Basic fertilization was supplemented with ^{15}N-labelled fertilizer, $(^{15}NH_2)_2CO$, $^{15}NH_4$-NO_3 and NH_4-$^{15}NO_3$, incorporated in soil.
- The quantity of ^{15}N isotope applied was 30 mg/pot.
- Foliar fertilizer treatments: 30 mL/pot (15 mL/plant) as 1% concentration solution.
- Irrigation 50% from the water pot capacity.
- Plants maintenance: daily irrigation up to 2/3 of the water capacity of the pot.
- 12 experimental variants; 3 replicates, 2 plants/pot, for each of the experimental factors combination (experimental variants).
- The control was basic and foliar fertilized.
- Harvesting maize biomass was done 60 days after seeding.

Table 1. Experimental diagram and types of fertilization

TYPES OF FERTILIZATION		
	RADICULAR	**FOLIAR**
BASIC	^{15}N-labeled fertilizer	
NPK 15-15-15 (nutrients dose $N_{45}P_{45}K_{45}$)	—	
	UREA (^{15}N-NH_2)	NUTRIFERT PLUS
	AMMONIUM NITRATE (^{15}N-NO_3)	
	AMMONIUM NITRATE (^{15}N-NH_4)	
	—	
	UREA (^{15}N-NH_2)	NUTRIFERT VEGETAL
	AMMONIUM NITRATE (^{15}N-NO_3)	
	AMMONIUM NITRATE (^{15}N-NH_4)	
	—	
	UREA (^{15}N-NH_2)	NUTRIFERT
	AMMONIUM NITRATE (^{15}N-NO_3)	
	AMMONIUM NITRATE (^{15}N-NH_4)	

The experimental data were statistically processed by variance analysis using the Duncan multiple comparison test, multiple comparison threshold α=0.05. For the graphic and statistical processing, the Excell, XLSTAT and SPSS 14.0 programs were used.

3. Results and discussions

The effects induced by combining the experimental factors on isotopic indicators ($^{15}N/^{14}N$ isotopic ratio, degree isotopic accumulation $\delta^{15}N$ in the maize plant, export of ^{15}N isotope with maize plant, ^{15}N isotope concentration in the maize plant, absorption degree of ^{15}N isotope from soil in maize plant) were evaluated in maize plant.

The experiment was bifactorial with two factors:

- A - chemical nature of the ^{15}N-labeled nitrogen from the fertilizers applied radicular; Graduations: a_1 Control; a_2 ^{15}N-NH_2; a_3 ^{15}N-NO_3; a_4 ^{15}N-NH_4

a_1 b_1 Control+Nutrifert	a_1 b_2 Control+Nutrifert Vegetal	a_1 b_3 Control+Nutrifert Plus
a_2 b_1 ^{15}N-NH_2+Nutrifert	a_2 b_2 ^{15}N-NH_2+Nutrifert Vegetal	a_2 b_3 ^{15}N-NH_2+Nutrifert Plus
a_3 b_1 - ^{15}N-NO_3 Nutrifert	a_3 b_2 ^{15}N-NO_3+Nutrifert Vegetal	a_3 b_3 ^{15}N-NO_3+Nutrifert Plus
a_4 b_1 ^{15}N-NH_4+Nutrifert	a_4 b_2 ^{15}N-NH_4+Nutrifert Vegetal	a_4 b_3 ^{15}N-NH_4+Nutrifert Plus

- B - chemical nature of the foliar fertilizers.

Graduations: b_1 Nutrifert; b_2 Nutrifert Vegetal; b_3 Nutrifert Plus. By combining the graduations of these two factors, AB, 12 variants were established.

Figure 2 shows the obtained values that were ordered in descending order resulting in a hierarchy of the effects of the application of the 12 experimental treatments. The highest values were:

- ammonium nitrate ^{15}N-NH_4+Nutrifert Vegetal (3,08%)
- ammonium nitrate ^{15}N-NH_4+Nutrifert Plus (3,07%)
- ammonium nitrate ^{15}N-NH_4+Nutrifert (3,04%)

Values ranked in the 1-3 hierarchy do not differ statistically from each other.

Ammonium nitrate ^{15}N-NH_4 (radicular applied) generated the highest values for $^{15}N/^{14}N$ isotopic ratio in the maize plant.

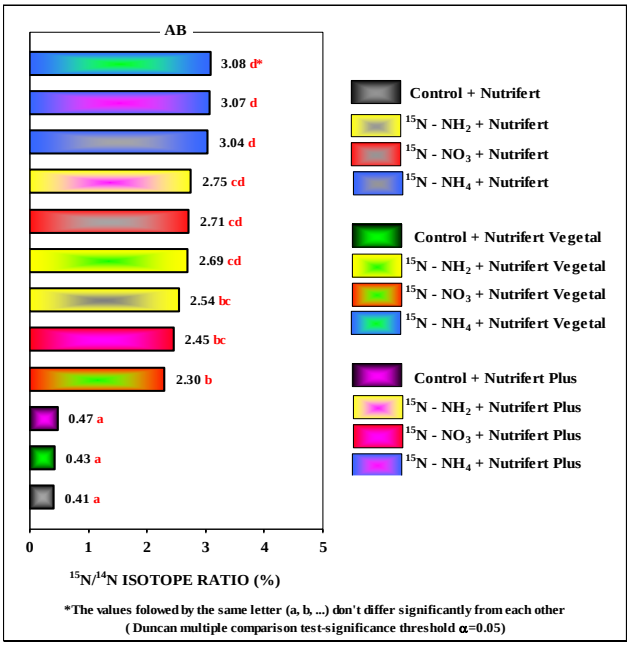

Figure 2. Effects induced by combining the chemical nature of radicular nitrogen source (A) with foliar fertilizers (B) on $^{15}N/^{14}N$ isotopic ratio in the maize plant

Figure 3 shows the obtained values that were ordered in descending order resulting in a hierarchy of the effects of the application of the 9 experimental treatments. The highest values were:

- ammonium nitrate ^{15}N-NH_4+Nutrifert Vegetal (7728‰)
- ammonium nitrate ^{15}N-NH_4+Nutrifert Plus (7699‰)
- ammonium nitrate ^{15}N-NH_4+Nutrifert (7616‰)

Values ranked in the 1-3 hierarchy do not differ statistically from each other.

Ammonium nitrate ^{15}N-NH_4 (radicular applied) generated the highest values degree for isotopic accumulation $\delta^{15}N$ in the maize plant.

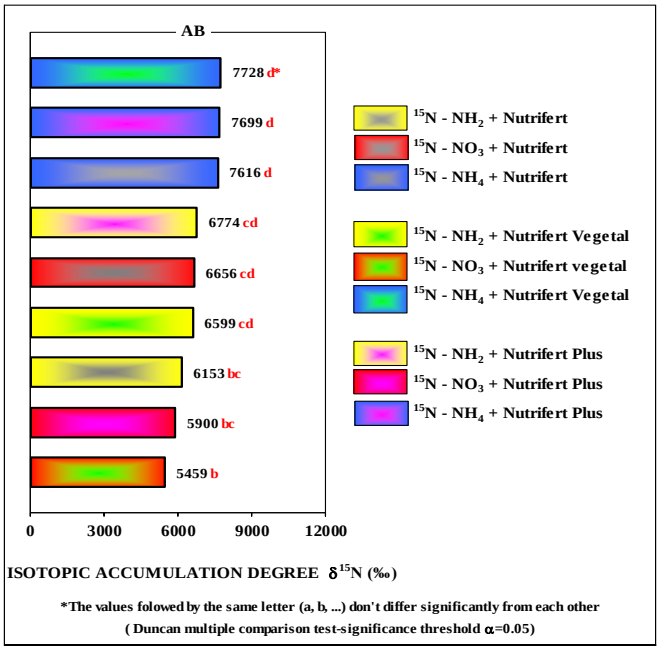

Figure 3. Effects induced by combining the chemical nature of radicular nitrogen source (A) with foliar fertilizers (B) on degree isotopic accumulation $\delta^{15}N$ in the maize plant

Figure 4 shows the obtained values that were ordered in descending order resulting in a hierarchy of the effects of the application of the 12 experimental treatments. The highest values were:

- urea ^{15}N-NH_2+ Nutrifert Plus (0,0036%)
- ammonium nitrate ^{15}N-NO_3+ Nutrifert Plus (0,0036%)
- urea ^{15}N-NH_2+ Nutrifert Vegetal (0,0035%)

Values ranked in the 1-3 hierarchy do not differ statistically from each other.

Urea ^{15}N-NH_2 and ammonium nitrate ^{15}N-NO_3 (radicular applied) generated the highest values for concentration ^{15}N isotope in the maize plant.

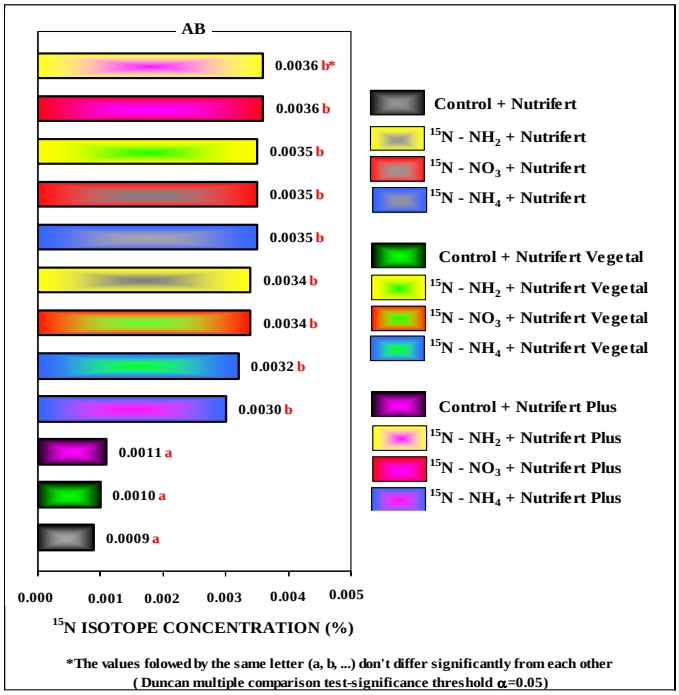

Figure 4. Effects induced by combining the chemical nature of radicular nitrogen source (A) with foliar fertilizers (B) on concentration the ^{15}N isotope in the maize plant

Figure 5 shows the obtained values that were ordered in descending order resulting in a hierarchy of the effects of the application of the 12 experimental treatments. The highest values were:

- urea ^{15}N-NH_2 + Nutrifert Plus (9,34 mg)
- urea ^{15}N-NH_2 + Nutrifert Vegetal (8,94 mg)
- ammonium nitrate ^{15}N-NO_3 + Nutrifert Plus (8,87 mg)

Values ranked in the 1-3 hierarchy do not differ statistically from each other.

Urea ^{15}N-NH_2 (radicular applied) generated the highest values for ^{15}N isotope export with the maize plant.

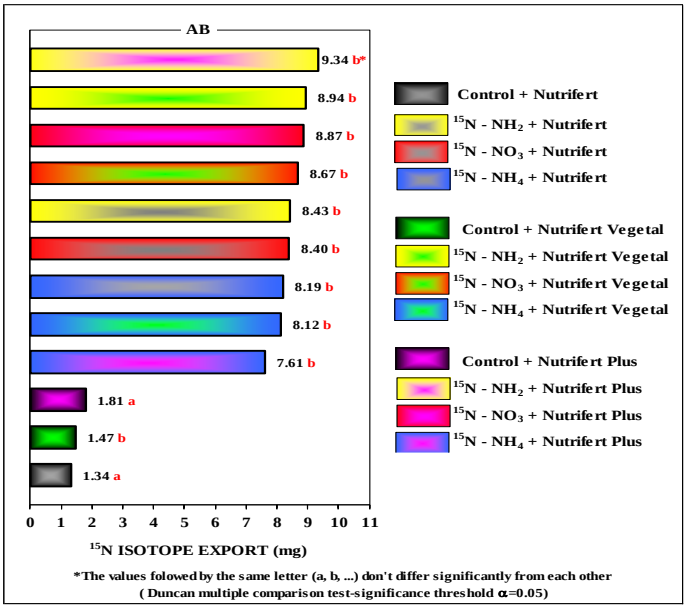

Figure 5. Effects induced by combining the chemical nature of radicular nitrogen source (A) with foliar fertilizers (B) on ^{15}N isotope export with the maize plant

Figure 6 shows the obtained values that were ordered in descending order resulting in a hierarchy of the effects of the application of the 9 experimental treatments. The highest value was: urea ^{15}N-NH_2 + Nutrifert Plus (26,0%)

Values obtained in the hierarchy of this 9 treatments do not differ statistically from each other. Urea ^{15}N-NH_2 (radicular applied) generated the highest values for absorption degree of ^{15}N isotope from soil in maize plant.

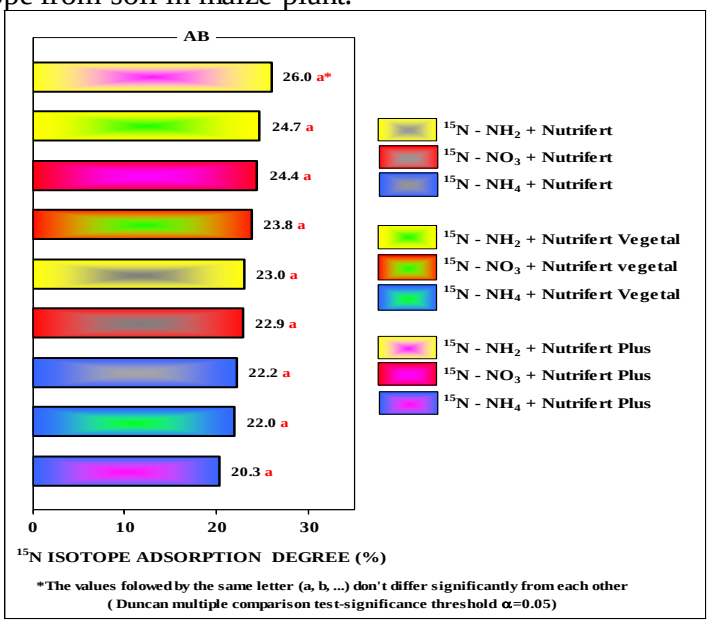

Figure 6. Effects induced by combining the chemical nature of radicular nitrogen source (A) with foliar fertilizers (B) on absorption degree of ^{15}N isotope from soil in maize plant

Conclusions

1. Four experimental foliar fertilizers (**NUTRIFERT NUTRIFERT, NUTRIFERT PLUS, NUTRIFERT ECO**) with complex matrix type N and NPK, with secondary nutrients, trace elements and natural organic substances with a chelating and biostimulating role were obtained.
2. The values of $^{15}N/^{14}N$ isotopic ratio, values of concentration ^{15}N isotope in maize plant and values of ^{15}N isotope export with the maize plant, that were obtained as a result of treatments including radicular fertilization with ^{15}N-labeled fertilizers and foliar fertilization, differ statistically significantly when compared to the values obtained for Control.
3. The degree of isotopic accumulation $\delta^{15}N$ values in the maize plant, obtained as a result of treatments including radicular fertilization with ^{15}N-labeled fertilizers and foliar fertilization, differs statistically significantly between them.
4. The values of absorption degree of ^{15}N isotope from soil in maize plant do not differ statistically significantly between them.
5. The highest values for $^{15}N/^{14}N$ isotopic ratio and degree of isotopic accumulation $\delta^{15}N$ in the maize plant were generated by the combined effects of radicular application of ammonium nitrate ^{15}N-NH_4 with foliar fertilizers.
6. The highest values for the ^{15}N isotope concentration in maize plant were generated by the combined effects of radicular application of ammonium nitrate ^{15}N-NO_3 with Nutrifert as foliar fertilizer and urea ^{15}N-NH_2 with Nutrifert Vegetal, respectively and Nutrifert Plus as foliar fertilizers.
7. The highest values for ^{15}N isotope export with the maize plant were generated by the combined effects of radicular application of urea ^{15}N-NH_2 with Nutrifert Vegetal, respectively and Nutrifert Plus as foliar fertilizers.
8. The chemical nature of the three foliar fertilizers Nutrifert, Nutrifert Vegetal, Nutrifert Plus did not affect the values obtained for ^{15}N isotopic indicators in maize plants.

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