

## ASSESSMENT OF THE EFFECTS OF SLOW-RELEASE FERTILIZERS APPLICATION OVER THE AMOUNT OF NITROGEN LEACHED AND OF THE DEVELOPMENT OF WINTER WHEAT PLANTS IN CONTROLLED CLIMATE CONDITIONS

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### Abstract

*Slow-release fertilizers provide a steady supply of plant nutrients over an extended period of time. The objective of these investigations was to study what was the amount of nitrogen leached after the administration of nitrogen fertilizers and how it influenced the development of plants during the vegetation period. The experiment was carried out in pots of vegetation with winter wheat. The experiment was conducted in the green house with controlled climate conditions within ICAM Iasi - USV Iasi. This paper presents the results obtained regarding the influence of 5 nitrogen fertilizers, 2 classic fertilizers and 3 slow-release nitrogen fertilizers. The experiment was carried out in pots of vegetation with winter wheat. The amount of nitrogen leached after the administration of nitrogen fertilizers is influenced by the chemical forms of nitrogen included in the chemical composition of the fertilizers, but also by the structure, texture, chemistry of the soil and its fertility status. The amount of nitrogen leached is significantly lower for Sulfammo 25 and 30 variants compared to N2017TAR, Ammonium Nitrate, Urea and the control, for both experiments.*

**Key words:** slow-release fertilizers, traditional fertilizer, nitrogen leached, soil chemistry.

### INTRODUCTION

The efficient use of nutrients by plants is highly dependent on the correct management of chemical fertilizers. The type of fertilizer applied can have a significant effect on crop yield (Stevens et al., 2005). Optimal fertilizer management must take into account the four pillars of fertilization: fertilizer type, optimal dose, timing of application, and the crop for which the fertilizer is intended (Volf & Raus, 2023).

Nitrogen-based fertilizers, although essential for crop development, exhibit a major drawback: more than three-quarters of their nutrient content is lost before being absorbed by plants. Urea and ammonium nitrate, the most frequently used nitrogen sources, rapidly degrade into ammonia - which evaporates into the atmosphere - and nitrates, which are highly susceptible to leaching. To compensate for these substantial nutrient losses, farmers are compelled to apply additional fertilizer quantities, consequently increasing production costs and reducing the overall economic efficiency of crops (Houlès et al., 2004; Laurent & Mary, 1992).

The extent of nitrogen loss following the application of nitrogen fertilizers depends on both the chemical form of nitrogen contained in the fertilizer and specific soil characteristics, including soil structure, texture, chemical properties, and overall fertility (Constantin et al., 2010; Addiscott, 2004).

Under typical conditions, nitrogen losses due to leaching can range between 10% and 30% of the total amount applied (Meisinger & Delgado, 2002). However, under adverse conditions - such as heavy rainfall, sandy soils, or excessive fertilization - nitrogen losses can rise significantly, reaching up to 50% (Baligar et al., 2001). On sandy soils, even under standard conditions, these losses are typically higher, ranging from 30% to 50%, and can further increase significantly with intensive irrigation or high applications of conventional nitrogen fertilizers associated with intensive agriculture (Beaudoin et al., 2005). Among the various nitrogen fertilizers, those containing nitrogen in nitrate form ( $\text{NO}_3^-$ ) have the highest susceptibility to leaching compared to fertilizers based on ammonium ( $\text{NH}_4^+$ ) or urea (Addiscott, 2004).

Through leaching, nitrates migrate from the soil into groundwater, thereby contaminating both soil and groundwater drinking sources. Nitrate concentrations often exceed the maximum permissible limit of 50 mg NO<sub>3</sub><sup>-</sup>/L (Directive (EU) 2020/2184). The quantity of nitrate entering the soil via leaching is substantial, commonly ranging from 50 to 150 kg NO<sub>3</sub><sup>-</sup>/ha/year or even higher (Beaudoin et al., 2005).

Selecting appropriate chemical fertilizers and ensuring correct application practices can substantially mitigate nitrogen losses through leaching, typically maintaining them below the 15-20% threshold. This approach considerably reduces environmental impacts and leads to significant economic savings (Constantin et al., 2010).

An increasingly adopted modern and effective strategy for reducing nitrogen losses and enhancing fertilizer efficiency is the use of slow-release or controlled-release fertilizers. The popularity and implementation of these advanced fertilizer formulations have grown markedly in recent years (Robbins, 2005).

Slow-release chemical fertilizers are formulated to gradually release nutrients into the soil over an extended period. This controlled release ensures a steady nutrient supply to plants throughout the growing season. As a result, nutrient concentrations in the soil solution remain at moderate levels, preventing excessive leaching of nitrogen and phosphorus into groundwater or surface water sources (Liu et al., 2020). This mechanism significantly reduces the risk of water pollution, particularly eutrophication, caused by fertilizer runoff. Additionally, ammonia volatilization and denitrification - the release of nitrogen gas from the soil - are lower compared to conventional readily available fertilizers, as high soil nitrogen concentrations do not accumulate over short periods.

The use of these fertilizers can reduce nitrogen losses through leaching by 30-50%. Under conditions such as sandy soils, high rainfall, or intensive irrigation, reductions in leaching losses can be even more substantial, reaching 40-60% (Trenkel, 2021; Guertal, 2009). Consequently, nutrient use efficiency, particularly nitrogen utilization, is significantly improved. Furthermore, by mitigating nitrogen

losses through leaching or volatilization, the overall efficiency of nitrogen uptake from chemical fertilizers is enhanced (Morgan et al., 2009).

For farmers, the adoption of these fertilizers allows for a reduction in the number of fertilization applications per season. Since nutrients are released gradually over an extended period, certain intermediate fertilization steps can be eliminated. This reduction in applications translates into time savings, lower labor requirements, and decreased fuel consumption costs (Volf & Raus, 2023; Liu et al., 2014).

Research indicates that the use of controlled-release fertilizers can achieve crop yields comparable to those obtained with conventional fertilizers but with 20-30% lower total nitrogen application rates (Liu et al., 2020). As a result, farmers can decrease the amount of fertilizer applied while maintaining similar yields, leading to significant financial savings and a reduction in negative environmental impacts (Guertal, 2009; Morgan et al., 2009).

## **MATERIALS AND METHODS**

Slow-release fertilizers primarily contain essential macronutrients - nitrogen (N), phosphorus (P), and potassium (K) - with nitrogen often receiving greater emphasis, typically in the form of urea or other nitrogen-based salts. In addition to N, P, and K, many slow-release formulations may also incorporate micronutrients such as iron, zinc, manganese, boron. However, the overall concentration of fertilizing elements in these products is generally lower compared to conventional concentrated fertilizers.

The basic chemical formula of the nutrients is similar to that of conventional fertilizers, including compounds such as urea, ammonium phosphates, sulphates or potassium chlorides. The main difference lies in the mechanism controlling solubilization. Various technologies have been developed in the fertilizer industry, leading to the classification of fertilizers according to their mechanism of action. Thus, there are slow-release fertilizers, controlled-release fertilizers and mixtures of active substances with different solubilization rates. The difference between these categories is

determined by how the active substance is released into the soil.

The primary objective of this study was to conduct a comparative analysis of the amount of nitrogen leached following fertilizer application and its influence on plant development throughout the growing season. To achieve this, five types of fertilizers with distinct characteristics were tested, considering factors such as chemical composition, the forms of nitrogen present in their formulations, solubilization degree, nutrient release mechanism in the soil, and effectiveness in minimizing leaching losses (Table 1).

Table 1. Characterization of tested fertilizers

| Fertilizers                 | Solubility in water at 20°C (g/L) | Chemical composition                                                                             | Specifications                                                   | Dissolution rate |
|-----------------------------|-----------------------------------|--------------------------------------------------------------------------------------------------|------------------------------------------------------------------|------------------|
| T1 Sulfammo 25              | 600                               | 25% Nt<br>18% NH <sub>4</sub><br>7% NO <sub>3</sub><br>2% MgO<br>31% SO <sub>3</sub>             | MPPA DUO - Activated Poly-Phenolic Molecules XCK: Plant extract  | Medium           |
| T2 Sulfammo 30              | 550                               | 30% Nt<br>5% NH <sub>4</sub><br>25% Urea<br>3% MgO<br>15% SO <sub>3</sub><br>0.15% B<br>0.10% Zn | Organocalcium matrix Microbial activator N-PRO, enzyme activator | Low              |
| T3 N2017 TAR                | n/a                               | n/a                                                                                              | unspecified                                                      | to be determined |
| T4 AN 34 (ammonium nitrate) | 1180                              | 34% Nt<br>17% NH <sub>4</sub><br>17% NO <sub>3</sub>                                             | -                                                                | Very fast        |
| T5 Urea 46                  | 1080                              | 46% Nt<br>46% CO(NH <sub>2</sub> ) <sub>2</sub>                                                  | -                                                                | Fast             |

The experiment was conducted in a climate-controlled greenhouse at ICAM - USV Iași, as well as in temperature-regulated climate chambers, enabling precise monitoring of experimental parameters, including soil characteristics, humidity, temperature, and light intensity. Detailed information regarding the experimental parameters is provided in Table 2. The experiment was performed using vegetation pots, with fall wheat as the test species. Constant-volume vegetation pots were filled with two soil types exhibiting distinct chemical properties (Table 3). The experimental design followed a randomized block design with four replications. Each vegetation pot was considered a single replicate, from which four soil and plant samples were collected and subsequently analyzed. The primary chemical

characteristic used in selecting the tested soil types was soil pH.

Table 2. Experimental parameters

| Conditions                           | Parameters                                   |
|--------------------------------------|----------------------------------------------|
| Vegetation pots                      |                                              |
| Pot diameter                         | 20 cm                                        |
| Soil column height                   | 20 cm                                        |
| Soil bulk density                    | 1.1 g/cm <sup>3</sup>                        |
| Pot surface                          | 314 cm <sup>2</sup>                          |
| Soil weight                          | 7 kg/pot                                     |
| Cultivated species                   | <i>Triticum aestivum</i> L., Ariesan variety |
| Sowing density                       |                                              |
| / pot                                | 18 seeds/pot                                 |
| / ha                                 | 500 no of seeds/m <sup>2</sup>               |
| Fertilizer application               | dissolved in 150 ml of distilled water       |
| N doses (equivalent/ha)              | 25 kg a.s. N/ha                              |
| T1 – Sulfammo 25                     | 0.38 g/pot                                   |
| T2 – Sulfammo 30                     | 0.31 g/pot                                   |
| T3 – N2017TAR                        | 0.39 g/pot                                   |
| T4 – AN 34                           | 0.28 g/pot                                   |
| T5 – Urea 46                         | 0.21 g/pot                                   |
| Low temperature simulation           | Climatic chamber                             |
| -1.1 / -2.9°C                        | 5 days                                       |
| Precipitation simulation             | BBCH 29                                      |
| Distilled water                      | 0.630 l/pot                                  |
| equivalent/m <sup>2</sup>            | 20 l/m <sup>2</sup>                          |
| Chlorophyll content – initial values | BBCH 10-12                                   |
| Chlorophyll content - stage I        | BBCH 25-26                                   |
| Chlorophyll content - stage II       | BBCH 35-37                                   |
| Total Nitrogen leachate - collector  | BBCH 29-30                                   |
| Total Nitrogen in plants             | BBCH 30-32                                   |

The physicochemical analysis of the soil samples was conducted in accordance with the ICPA Bucharest methodology, which serves as the standard protocol in all agrochemical laboratories across Romania. The following parameters were assessed: soil pH, total nitrogen, accessible phosphorus, accessible potassium, total calcium carbonate, and particle size distribution. To determine leached nitrogen, water samples were collected from the designated collectors. To determine the total nitrogen content in water samples, the STAS 7184/2-85 standard method was applied. This method involves a wet oxidation process using concentrated sulfuric acid and a catalyst to digest nitrogen-containing compounds into ammonium, followed by a colorimetric determination using Nessler's reagent or phenol-hypochlorite reaction, depending on the detection system. The absorbance is measured spectrophotometrically, and nitrogen concentration is calculated based on a calibration curve. Plant growth analysis included the determination of total nitrogen content and protein content

using the Kjeldahl method according to SR ISO 3188:1995. The method involves acid digestion of plant material with sulfuric acid and a catalyst, followed by distillation and titration to quantify the nitrogen content, which was then converted to protein using a standard factor.

Chlorophyll content was assessed using a Chlorophyll Content Meter (CCM-200 plus, Opti-Sciences, USA), which estimates relative chlorophyll concentration non-destructively by measuring light transmittance at two wavelengths (red and near-infrared) through the leaf tissue. Stomatal conductance was measured with a SC-1 Leaf Porometer (Decagon Devices, USA), which determines the rate of water vapor diffusion through the stomata, providing an indirect indicator of plant water status and gas exchange capacity.

Statistical analysis was performed using SPSS software, applying analysis of variance (ANOVA). Differences between variants were statistically evaluated using Duncan's test ( $p < 0.05$ ).

## RESULTS AND DISCUSSIONS

### Soil characterization in vegetation pots

It is well established that soil reaction (pH) significantly influences both the fixation and availability of nitrogen from fertilizers, exerting a major impact on biological and chemical processes within the soil.

The retention of nitrogen forms from fertilizers varies depending on soil pH. Ammoniacal nitrogen ( $\text{NH}_4^+$ ) is more effectively fixed in acidic or neutral soils (pH 5.5–7), where it is adsorbed onto clay and humus particles. In contrast, in alkaline soils (pH >7.5),  $\text{NH}_4^+$  is converted into gaseous  $\text{NH}_3$ , leading to volatilization losses. Nitric nitrogen ( $\text{NO}_3^-$ ) remains highly mobile and is poorly retained in soils regardless of pH, making it particularly susceptible to leaching in acidic and permeable soils, especially at pH levels below 5.5. Amide nitrogen (urea,  $\text{CO}(\text{NH}_2)_2$ ) undergoes rapid hydrolysis to  $\text{NH}_4^+$ ; however, at pH levels above 7, this process is accompanied by the volatilization of gaseous ammonia ( $\text{NH}_3$ ) (Volf & Raus, 2023).

Based on these considerations, experiments were conducted on two soil types with distinct chemical properties, the compositions of which are detailed in Table 3.

In this context, two experiments were designed, maintaining all factors constant except for soil type and its corresponding pH. The first experiment utilized an alkaline soil (pH = 8.38), characterized by high phosphorus and potassium content, moderate nitrogen levels, and a fine silt texture. The second experiment employed a neutral soil (pH = 7.04), which was well-supplied with potassium, had moderate nitrogen and phosphorus levels, and exhibited a texture closely resembling that of the soil used in the first experiment (Table 3).

Table 3. Physico-chemical soil characterization

| Soil analysis                | Units | Soil 1      |                     | Soil 2      |                 |
|------------------------------|-------|-------------|---------------------|-------------|-----------------|
|                              |       | Mean values | Characteristics     | Mean values | Characteristics |
| pH                           | -     | 8.38        | Moderate alkalinity | 7.04        | Neutral         |
| Total Nitrogen               | %     | 0.193       | Medium              | 0.149       | Medium          |
| Available Phosphorus         | ppm   | 78          | Very high           | 30          | Medium          |
| Accessible Potassium         | ppm   | 498         | Very high           | 331         | Very high       |
| Total calcium carbonate      | %     | 4.16        |                     | 0.63        |                 |
| Soil texture                 |       |             | Medium texture      |             | Medium texture  |
| Coarse sand (2.0-0.2 mm)     | %     | 1.0         | Fine silt           | 0.8         | Fine silt       |
| Fine sand (0.2-0.02 mm)      | %     | 31.2        |                     | 34.5        |                 |
| Silt (0.02-0.002 mm)         | %     | 38.1        |                     | 29.3        |                 |
| Clay (< 0.002 mm)            | %     | 29.5        |                     | 35.4        |                 |
| Macroscopic clay (< 0.01 mm) | %     | 49.9        |                     | 52.4        |                 |

### Leached Total Nitrogen (LTN)

The amount of nitrogen leached from nitrogen fertilizer applications is influenced by both the chemical forms of nitrogen present in the fertilizer composition and soil properties,

including structure, texture, chemical composition, and fertility status.

The quantity of leached nitrogen was significantly lower for Sulfammo 25 and Sulfammo 30 compared to N2017TAR, AN 34, Urea 46,

and the control in both trials. Ammoniacal nitrogen ( $\text{NH}_4^+$ ) was more efficiently retained in neutral soil ( $\text{pH} = 7.04$ ), where it was adsorbed onto clay and humus particles. In contrast, unprotected urea was rapidly solubilized, leading to a significant loss of total nitrogen (Table 4). Due to the chemical composition of Sulfammo 25 and Sulfammo 30, as well as the presence of ammonium ions ( $\text{NH}_4^+$ ), which have a high adsorption capacity on the colloidal complex and low mobility within the soil, the total nitrogen content in the leached water was lower compared to the other treatments. This can be attributed to the fact that the other fertilizer variants contained nitrogen in the form of nitrate ( $\text{NO}_3^-$ ), which, due to its negative ionic charge and high mobility, is more prone to leaching. In alkaline soil, nitrogen losses in the form of nitrate were more pronounced, resulting in higher levels of total leached nitrogen (Table 4). The greater amount of leached nitrogen in the N2017TAR and Urea 46 treatments can also be explained by the high content of amide nitrogen (N-amide), which is susceptible to leaching through the soil profile due to its low polarization. Additionally, the conversion of N-amide in the soil occurred rapidly due to the high temperatures recorded during the test period, which accelerated its transformation into nitric nitrogen (N-nitric). Other factors, such as soil texture, structure, and organic matter content, also influenced the rate of nitrogen leaching.

Table 4. Leached Total Nitrogen (LTN, % DM)

| Treatments      | LTN (%)<br>Alkaline Soil pH | LTN (%) Neutral<br>Soil pH |
|-----------------|-----------------------------|----------------------------|
| T1 Sulfammo 25% | 0.256 <sup>a</sup>          | 0.398 <sup>a</sup>         |
| T2 Sulfammo 30% | 0.455 <sup>a</sup>          | 0.512 <sup>a</sup>         |
| T3 N2017TAR     | 0.796 <sup>b</sup>          | 0.568 <sup>a</sup>         |
| T4 AN 34%       | 0.810 <sup>b</sup>          | 0.682 <sup>ab</sup>        |
| T5 Urea 46%     | 0.824 <sup>b</sup>          | 0.896 <sup>b</sup>         |
| T6 Unfertilized | 0.106 <sup>c</sup>          | 0.114 <sup>c</sup>         |

<sup>1</sup> Values followed by the same letter in the exponent are not significantly different at  $p \leq 0.05$  (Duncan test)  
<sup>2</sup> DM = Dry Matter  
<sup>3</sup> DM = 88.0%

### Total Nitrogen (TN) and Crude Protein (CP) Content in Plants

The total nitrogen (TN) content in the dry matter was higher for Sulfammo 25, Sulfammo 30, and N2017TAR compared to the other fertilizers. This can be attributed to the greater amount of solubilized nitrogen remaining in plant-available forms (Table 5).

The differences in total nitrogen content between the two trials were influenced by variations in the pH of the nutrient medium. It is well established that the uptake intensity of  $\text{NH}_4^+$  increases with pH, with neutral pH conditions favoring its absorption. In alkaline soils, the total nitrogen (TN) content, expressed as a percentage of dry matter, falls within a very low safety class according to the ICPA scale, which may lead to nitrogen deficiency across all fertilization treatments. Conversely, in neutral pH soils, the TN content was 3.63% for the Sulfammo 25 treatment (classified as low assurance) and 5.86% for the Sulfammo 30 treatment (classified as normal assurance) (Table 5). These findings suggest that, when Sulfammo 30 was applied, the ammoniacal nitrogen contained in the fertilizer remained available in the soil for a longer period, was translocated more efficiently within the plant, and was optimally metabolized.

Table 5. Total Nitrogen (TN) and Crude Protein (CP) Content in Plants Expressed on a Dry Matter Basis (% DM)

| Treatments      | TN (%)<br>Alkaline<br>Soil pH | CP (%)<br>Alkaline<br>Soil pH | TN (%)<br>Neutral<br>Soil pH | CP (%)<br>Neutral<br>Soil pH |
|-----------------|-------------------------------|-------------------------------|------------------------------|------------------------------|
| T1 Sulfammo 25% | 2.09 <sup>a</sup>             | 13.06 <sup>a</sup>            | 3.63 <sup>ab</sup>           | 22.69 <sup>a</sup>           |
| T2 Sulfammo 30% | 2.21 <sup>a</sup>             | 13.81 <sup>a</sup>            | 5.86 <sup>a</sup>            | 36.63 <sup>a</sup>           |
| T3 N2017TAR     | 1.93 <sup>b</sup>             | 12.06 <sup>a</sup>            | 4.90 <sup>a</sup>            | 30.63 <sup>a</sup>           |
| T4 AN 34%       | 1.13 <sup>b</sup>             | 7.06 <sup>b</sup>             | 3.45 <sup>ab</sup>           | 27.81 <sup>a</sup>           |
| T5 Urea 46%     | 1.36 <sup>b</sup>             | 8.50 <sup>b</sup>             | 2.16 <sup>b</sup>            | 13.50 <sup>b</sup>           |
| T6 Unfertilized | 1.20 <sup>b</sup>             | 4.75 <sup>b</sup>             | 1.63 <sup>c</sup>            | 11.69 <sup>b</sup>           |

<sup>1</sup> Values followed by the same letter in the exponent are not significantly different at  $p \leq 0.05$  (Duncan test)  
<sup>2</sup> DM = Dry Matter  
<sup>3</sup> DM = 88.0%  
<sup>4</sup> The results for TN and CT are expressed on a DM basis.

### Effect of Different Treatments on Stomatal Conductance (SC)

Stomatal conductance (SC) was measured to assess plant water use efficiency and its impact on photosynthesis. Chemical fertilizers, particularly nitrogen-based fertilizers, play a crucial role in plant metabolism by influencing soil water and nutrient uptake, enhancing resistance to external stresses, promoting leaf mass development, and ultimately affecting yield. However, no significant differences were observed between fertilization treatments. Soil pH influences stomatal conductance (SC) through multiple mechanisms, affecting nutrient availability, plant hormone balance, and root system health. All tested fertilizers resulted in higher stomatal conductance values in neutral



pH soils compared to alkaline pH soils (Table 6). An optimal pH (typically between 6 and 7) supports efficient stomatal function, maximizing gas exchange and photosynthesis. In contrast, alkaline soil conditions can induce physiological stress in plants by stimulating the synthesis of abscisic acid (ABA), which triggers stomatal closure to reduce water loss.

Table 6. Effect of Different Treatments on Stomatal Conductance (SC, mmol m<sup>-2</sup> s<sup>-1</sup>) under Alkaline and Neutral Soil pH Conditions

| Treatments      | SC (mmol m <sup>-2</sup> s <sup>-1</sup> )<br>Alkaline Soil pH | SC (mmol m <sup>-2</sup> s <sup>-1</sup> )<br>Neutral Soil pH |
|-----------------|----------------------------------------------------------------|---------------------------------------------------------------|
| T1 Sulfammo 25% | 31.2 <sup>a</sup>                                              | 68.8 <sup>a</sup>                                             |
| T2 Sulfammo 30% | 31.3 <sup>a</sup>                                              | 68.0 <sup>a</sup>                                             |
| T3 N2017TAR     | 29.4 <sup>a</sup>                                              | 64.9 <sup>a</sup>                                             |
| T4 AN 34%       | 31.2 <sup>a</sup>                                              | 49.0 <sup>a</sup>                                             |
| T5 Urea 46%     | 31.6 <sup>a</sup>                                              | 55.6 <sup>a</sup>                                             |
| T6 Unfertilized | 49.7 <sup>b</sup>                                              | 68.8 <sup>a</sup>                                             |

<sup>1</sup>Values followed by the same letter in the exponent are not significantly different at p ≤ 0.05 (Duncan test)

Effect of Different Treatments on Chlorophyll Content (CCI)

Soil pH influences plant chlorophyll content by regulating the availability of essential nutrients involved in chlorophyll synthesis and photosynthetic processes. An imbalanced pH can lead to mineral deficiencies, negatively affecting both the quantity and stability of chlorophyll in leaves. The dynamics of chlorophyll content exhibited distinct trends in the two experiments due to differences in the pH of the nutrient medium. An alkaline pH environment facilitated the uptake of NH<sub>4</sub><sup>+</sup> from fertilizers with a high nitrogen content in this form (Table 7).

Table 7. Effect of Different Treatments on Chlorophyll Content (Chlorophyll Content Index, CCI units) under Alkaline Soil pH Conditions

| Treatments      | CCI –<br>BBCH 10-<br>12 | CCI –<br>BBCH 25-<br>26 | CCI –<br>BBCH 35-<br>37 |
|-----------------|-------------------------|-------------------------|-------------------------|
| T1 Sulfammo 25% | 5.0 <sup>a</sup>        | 9.4 <sup>a</sup>        | 12.5 <sup>b</sup>       |
| T2 Sulfammo 30% | 5.3 <sup>a</sup>        | 11.0 <sup>ab</sup>      | 15.3 <sup>b</sup>       |
| T3 N2017TAR     | 4.3 <sup>a</sup>        | 11.7 <sup>ab</sup>      | 11.1 <sup>ab</sup>      |
| T4 AN 34%       | 4.2 <sup>a</sup>        | 13.9 <sup>b</sup>       | 9.9 <sup>ab</sup>       |
| T5 Urea 46%     | 5.1 <sup>a</sup>        | 14.2 <sup>b</sup>       | 4.3 <sup>a</sup>        |
| T6 Unfertilized | 4.7 <sup>a</sup>        | 9.8 <sup>b</sup>        | 8.7 <sup>ab</sup>       |

<sup>1</sup>Values followed by the same letter in the exponent are not significantly different at p ≤ 0.05 (Duncan test)  
<sup>2</sup>Number of determinations – 15 (weighted average: base/middle/top – 5 plants – first leaf)

The form of nitrogen present in fertilizers significantly influences plant uptake, metabolism,

and nitrogen content, directly affecting crop growth, photosynthesis, and yield. A comparison of the experimental variants revealed that, during the early stages of the growing season, chlorophyll content was lower in the Sulfammo 25, Sulfammo 30, and N2017TAR treatments compared to AN 34 and Urea 46, despite the higher total nitrogen content in the plants. As the growing season progressed, chlorophyll content increased in the Sulfammo 25, Sulfammo 30, and N2017TAR treatments, surpassing the values recorded for AN 34 and Urea 46.

This phenomenon can be attributed to the slower uptake of NH<sub>4</sub><sup>+</sup>, which remained available to plants over an extended period. The reduced uptake rate of this nitrogen form facilitated a more balanced development between leaves and roots, promoted a higher protein content, and consequently led to a later increase in chlorophyll content. While Urea 46 provided a balance between the effects of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, it was also associated with high nitrogen losses (Table 8).

Table 8. Effect of Different Treatments on Chlorophyll Content (Chlorophyll Content Index, CCI units) Under Neutral Soil pH Conditions

| Treatments      | CCI –<br>BBCH 10-<br>12 | CCI –<br>BBCH 25-<br>26 | CCI –<br>BBCH 35-<br>37 |
|-----------------|-------------------------|-------------------------|-------------------------|
| T1 Sulfammo 25% | 1.2 <sup>a</sup>        | 3.3 <sup>bc</sup>       | 3.3 <sup>a</sup>        |
| T2 Sulfammo 30% | 1.2 <sup>a</sup>        | 2.6 <sup>abc</sup>      | 3.1 <sup>a</sup>        |
| T3 N2017TAR     | 2.0 <sup>a</sup>        | 2.5 <sup>ab</sup>       | 3.6 <sup>a</sup>        |
| T4 AN 34%       | 1.8 <sup>a</sup>        | 3.4 <sup>c</sup>        | 1.8 <sup>b</sup>        |
| T5 Urea 46%     | 2.1 <sup>a</sup>        | 3.2 <sup>bc</sup>       | 2.1 <sup>b</sup>        |
| T6 Unfertilized | 1.9 <sup>a</sup>        | 2.0 <sup>a</sup>        | -                       |

<sup>1</sup>Values followed by the same letter in the exponent are not significantly different at p ≤ 0.05 (Duncan test)  
<sup>2</sup>Number of determinations – 15 (weighted average: base/middle/top – 5 plants – first leaf)

Relationship Between Leached Total Nitrogen and Plant Total Nitrogen Content

An analysis of the relationship between leached total nitrogen (LTN, %) and total plant nitrogen content (TN, %) reveals that the fertilizers Sulfammo 25 and Sulfammo 30 exhibit the lowest LTN values (Figure 1). This suggests a higher nitrogen retention efficiency in the soil, thereby reducing nitrogen losses through leaching. Concurrently, these fertilizers contribute to the highest total nitrogen accumulation in plants, ensuring a sustained nitrogen supply throughout the growing season. Their effectiveness is particularly notable in moderately alkaline soils.

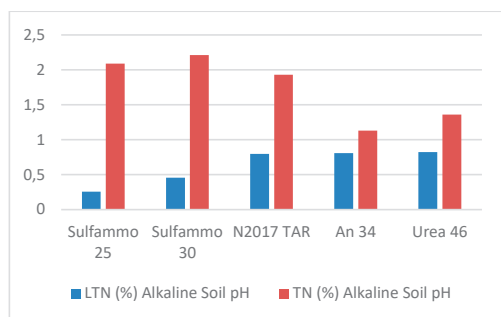


Figure 1. Relationship Between Leached Total Nitrogen and Plant Total Nitrogen Content in Alkaline Soil

Conversely, AN 34 and Urea 46 displayed the highest nitrogen leaching losses (LTN, %), indicating their potential suitability for soils with a high nutrient retention capacity or for application in controlled fertilization systems (Figure 2). Consequently, under the experimental conditions analyzed, the total nitrogen content in plants (TN, %) was significantly lower when these fertilizers were applied.

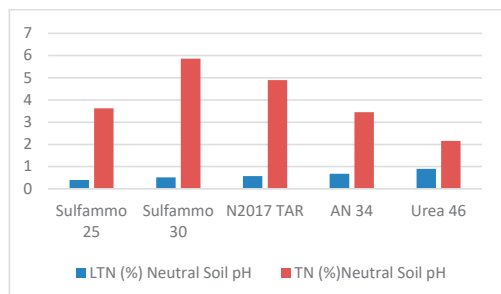


Figure 2. Relationship Between Leached Total Nitrogen and Plant Total Nitrogen Content in Neutral Soil

The analysis of data from neutral pH soils confirms the same ranking of fertilizer effectiveness as observed in alkaline pH soils. Sulfammo 30 and Sulfammo 25 emerged as the most effective fertilization options, demonstrating superior nitrogen uptake and minimized leaching losses. In contrast, AN 34 and N2017 TAR exhibited moderate total plant nitrogen content (TN, %). However, AN 34 experienced higher nitrogen leaching losses, which may contribute to reduced agronomic efficiency.

### Comparison of Chlorophyll Content and Total Plant Protein Content

Correlation analysis between crude protein content and chlorophyll content in alkaline-

reacting soils does not indicate a directly proportional relationship between these variables (Figure 3). However, Sulfammo 30 recorded the highest crude protein percentage (14%), suggesting a more efficient accumulation of nitrogen in plant proteins. In contrast, AN 34 exhibited the lowest crude protein content (6%), indicating a reduced conversion of nitrogen into plant protein. Although the variants fertilized with AN 34 and Urea 46 showed the highest chlorophyll content values, their crude protein levels remained low. This finding suggests that while AN 34 and Urea 46 enhance chlorophyll synthesis, they do not significantly contribute to protein accumulation. Consequently, the nitrogen supplied by these fertilizers appears to be preferentially utilized for photosynthetic metabolism rather than for protein biosynthesis.

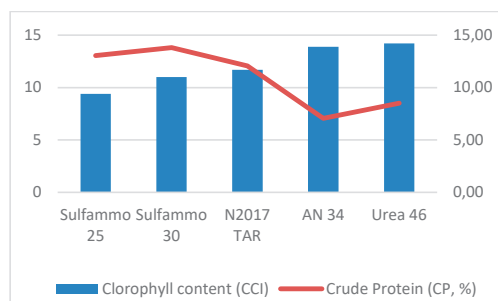


Figure 3. Relationship Between Chlorophyll Content and Total Plant Protein Content in Alkaline Soil

In neutral-reacting soils, AN 34 exhibited the highest chlorophyll content, followed by Urea 46 and N2017 TAR, suggesting that these fertilizers enhance the efficiency of photosynthetic processes (Figure 4).

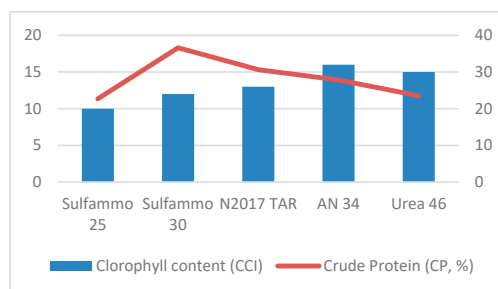


Figure 4. Comparison of Chlorophyll Content and Total Plant Protein Content in Neutral Soil

Conversely, Sulfammo 30 recorded the highest crude protein content (CP %) but did not achieve

the highest chlorophyll levels. This finding indicates that the nitrogen supplied by this fertilizer is utilized more efficiently for protein synthesis rather than for the production of photosynthetic pigments. N2017 TAR displayed intermediate values for both chlorophyll and crude protein content, suggesting a balanced allocation of nitrogen between these two physiological processes.

## CONCLUSIONS

### Chlorophyll and Total Protein Content: Relationship and Fertilizer Influence

The absence of a direct correlation between chlorophyll content and total protein content suggests that nitrogen utilization varies depending on the fertilizer type and the physiological requirements of the plant.

AN 34 and Urea 46 primarily stimulate chlorophyll synthesis but do not significantly contribute to protein accumulation. This observation indicates that the nitrogen supplied by these fertilizers is predominantly used for primary plant metabolism, including photosynthetic processes, rather than for protein biosynthesis. This phenomenon can be attributed to the rapid uptake of nitrate nitrogen ( $\text{NO}_3^-$ ), which accumulates in young plants, enhancing chlorophyll production but requiring additional metabolic conversion for protein synthesis.

Sulfammo 25 is particularly suited for alkaline soils or soils prone to high nitrogen leaching due to its slower nitrogen release. While it is more efficient in promoting crude protein accumulation than Urea 46 and AN 34, it does not reach the performance level of Sulfammo 30. Despite not stimulating chlorophyll synthesis to the same extent, Sulfammo 25 ensures an optimal balance between protein content and nitrogen use efficiency.

Among the fertilizers analyzed, Sulfammo 30 is the most effective in enhancing crude protein content, irrespective of soil pH. It achieves the highest CP (%) values, even though it does not result in the highest chlorophyll accumulation. The prolonged availability of ammonium nitrogen ( $\text{NH}_4^+$ ) allows plants to utilize nitrogen efficiently, facilitating metabolic processes involved in protein biosynthesis. However, the overall efficiency of this fertilizer is influenced by factors such as crop type, soil properties, and

microbiological activity, which regulate nitrogen transformations in the soil.

### Total Plant Nitrogen and Total Leached Nitrogen

Soils with a neutral pH facilitate nitrogen uptake, regardless of the type of fertilizer applied. The total plant nitrogen content (TN, %) was significantly higher in neutral soils compared to alkaline soils across all treatments. The most substantial improvements were observed for Sulfammo 30 and Sulfammo 25, indicating that these fertilizers are particularly effective under neutral soil conditions. Their high efficiency can be attributed to their ammoniacal nitrogen ( $\text{NH}_4^+$ ) content, which remains more stable in neutral soils and is less prone to losses through volatilization or leaching.

Intermediate but satisfactory TN (%) values were also recorded for N2017 TAR, which demonstrated greater effectiveness in neutral soils. This could be due to its composition, which likely includes both ammonium nitrogen ( $\text{NH}_4^+$ ) and nitrate nitrogen ( $\text{NO}_3^-$ ), providing greater adaptability in soils with variable pH. The improved nitrogen uptake in neutral soils is explained by the optimized nitrogen transformation processes and enhanced plant absorption.

### Nitrogen Leaching and Fertilizer Susceptibility

Nitrogen leaching did not vary significantly between alkaline and neutral soils. However, the highest leaching losses were recorded for AN 34 and Urea 46, demonstrating their susceptibility to nitrogen loss, regardless of soil pH. These fertilizers contain nitrogen primarily in nitrate form ( $\text{NO}_3^-$ ) or in forms that rapidly convert to  $\text{NO}_3^-$ , such as the rapid solubilization of urea, making them highly soluble and prone to leaching.

To minimize nitrogen losses, Sulfammo 25 and Sulfammo 30 are the most suitable fertilizers for both neutral and alkaline soils. These fertilizers contain nitrogen in ammoniacal ( $\text{NH}_4^+$ ) and amide ( $\text{CO}(\text{NH}_2)_2$ ) forms, which are more stable in the soil and less prone to leaching.

### Nitrogen Stabilization Technologies

To enhance nitrogen retention and reduce losses due to leaching or fixation, Sulfammo 25 and Sulfammo 30 incorporate advanced stabilization technologies, including:



MPPA DUO - Activated Poly-Phenolic Molecules (Sulfammo 25)

Organocalcium Matrix (Sulfammo 30)

These components provide superior nitrogen protection (as detailed in Table 1). Active polyphenols in MPPA DUO slow the conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  by partially inhibiting nitrification, thereby improving nitrogen retention in the soil and enhancing plant uptake. The Organocalcium Matrix stabilizes nitrogen through a combination of calcium and organic compounds, reducing leaching losses. Additionally, calcium influences soil pH and microbiological activity, thereby regulating the conversion rate of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ .

### Recommendation

On both soil types (neutral and alkaline), Sulfammo 25 and Sulfammo 30 exhibited the lowest nitrogen leaching losses, confirming their status as the most effective fertilizers for minimizing nitrogen loss. Moreover, neutral soils significantly enhance plant nitrogen uptake, with Sulfammo 30 emerging as the best-performing fertilizer under these conditions.

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