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EFFECTS OF DIFFERENT NITROGEN FERTILIZER RELEASE TYPES AND APPLICATION MOMENTS IN HYBRID SEED MAIZE PRODUCTION

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Abstract

The use of slow-release and controlled-release fertilizers (SRFs and CRFs) is aimed at providing a step towards better N management. Starting from this idea, the aim of the paper is to present the results of the performed research focused on analyzing and comparing the SRFs and CRFs to classical ones, in terms of yield and phenological and phenotypical development of plants in hybrid seed maize production under irrigated conditions, as well as in researching the optimum time of application for each kind of the studied fertilizer. Research has been conducted for 3 years (2020, 2021, and 2022) in irrigated fields located in NE Romania, the experimental factors being the following: Factor A – nitrogen fertilizer product (a1 and a2 = Control 1 and Control 2 – no added nitrogen fertilizer; a3 = ammonium nitrate; a4 = Urea NG; a5 = Multicote (4) 34-0-7); Factor B – fertilizer rate split between seedbed preparation (SbP) and BBCH 16 (b1=100% SbP; b2=50% SbP + 50% BBCH 16; b3=25% SbP + 75% BBCH 16; b4=100% BBCH 16). The obtained results sustain the use of SRFs and CRFs in hybrid seed maize production, which is leading to better yields compared to classical fertilizers, no negative impact on synchronization at pollination time, lower quantities of physical fertilizer product to be used, and reduced number of passes in the field. The best moment of application of nitrogen fertilizers in hybrid seed maize production differs according to fertilizer type: slow-release fertilizers are best applied all at seedbed preparation, controlled-release fertilizers are best applied in 50-50 split between seedbed preparation and BBCH 16 sidedressed, while ammonium nitrate should be applied at BBCH 16 with cultivation.

Key words: *Maize, hybrid seed, slow-release fertilizers, controlled release fertilizers, nitrogen, seed yield.*

INTRODUCTION

Climate change and the continuously and rapidly increase in population are two challenges which are at the forefront of agriculture nowadays and for the foreseeable future. Globally, crop production needs to be increased, upgraded, and safeguarded (Vejan et al., 2021), while reducing the environmental impact and preserving the soil health (Ghumman et al., 2022).

Nowadays, agricultural intensification is the main alternative that encourages farmers to increase agricultural production with limited agricultural land (Kaavessina et al., 2021) and fertilization, as an essential tool of intensification, is a basic technological element of modern agriculture (Șimon et al., 2022). The use of fertilizers is essential for plant growth (Negi et al., 2022), and the effective use of inorganic fertilizers has been responsible for increased food production more than any other input (Penuelas et al., 2023). Fertilizers provide nutrients to plants and they are credited for the improvement in crop yield, which results in exponentially increased fertilizer use worldwide (Lawrencia et al., 2021). Meanwhile, the massive use and misuse of mineral fertilizers resulted in land degradation and impairing the sustainability of many agro-ecosystems (Allam et al., 2021), this having a negative impact on the environment and exacerbating the effects of climate change (Benlamlih et al., 2021; Kaavessina et al., 2021). Thus, the aim of using fertilizers is to reduce the environmental impact and to enhance food quality while maintaining acceptable yields (Spiertz, 2010).

Increase in fertilizer prices coupled with the need to reduce negative ecological effects imposes necessity to have a better correlation between the moment of nutrient consumption and their application especially regarding nitrogen. Among all nutrients, nitrogen is the nutrient most limiting crop production in all areas of the world and is generally applied to soil in the largest quantity (Penuelas et al., 2023; Spiertz, 2010). About 50% of the increase in food grain production been attributed to fertilizer use and more than 30-40% increase is due to nitrogen fertilizers alone, but about one-fourth to one-third of the applied fertilizer nitrogen is lost as ammonium and nitrous oxide gases to the atmosphere or as nitrates to the surface or ground waters, creating several environmental and health problems (Bahar et al., 2019). Unfortunately, discretionary use of nitrogen fertilizers in the past, in high quantities, have favoured leaching of nitrogen into ground waters and high losses of nitrogen available for plant uptake (Wang et al., 2019; Goulding et al., 2008). Therefore, nitrogen management requires special attention in its use so that the losses can be minimized, and the efficiency maximized (Jat et al., 2012), especially in highly demanding crops where high N rates are used (Arrobas et al., 2022). Practically, it is necessary to optimize N fertilizer use both to meet crop requirements and to reduce N losses (Zheng et al., 2016).

Classical maize fertilization technology has been constructed by practicians around old products of chemical synthesis with 1:1:1 or 1:3:0 N:P:K ratios, urea or ammonium nitrate all which have been developed in the first half of the 20th century (Hergert et al., 2015). The nutrients from these types of fertilizers are assimilated by plants through the concept of solubilization and uptake through the root system, but unfortunately recent research has shown that these classical fertilizers are very sensitive to excess water, with the implied levigation, as well as to volatilization (Schwenke et al., 2022).

New types of fertilizers have been developed in the past decade to better copy the pattern of nitrogen uptake of crops, respectively slow-release fertilizers (SRF) and controlled-release fertilizers (CRF) which use either a polymer coating or nitrification inhibitors to model nitrogen release to follow more accurately the plant consumption. SRFs and CRFs provide a more efficient, economical, and safe way to deliver nutrients to plants as they can retain nutrients in the soil for a longer period and they are available for the plants at the desired rate or concentration level (Kontárová et al., 2022). The basic concept of these fertilizers is that they release nutrients to plants over an extended period, more efficiently than classic-release fertilizers (Khunkeaw et al., 2012), to synchronize with the nutrient demand curve of a crop during a production cycle. This can meet the crop nutrient demand for the entire season through a single application, involving savings in spreading costs (Shaviv, 2020). Thus, one-time fertilization with controlled-released fertilizer (CRF) is a promising way for reducing labor cost, increasing nitrogen use efficiency (NUE) and alleviating environmental pollution (Cui et al., 2022).

Slow-release nitrogen fertilizer (SRNF) can potentially increase crop production, improve NUE, reduce fertilizer nutrient loss, leaching and denitrification in the soil, and significantly reduce apparent nitrogen loss in the 0-90 cm soil layer (Bahar et al., 2019; Zheng et al., 2017; Feng et al., 2021), and thus reducing the environmental challenges in agro-ecosystem (Ghafoor et al., 2022). The goal in developing SRNF is having N release rated to the requirement of growing crop, thereby reducing the loss of applied fertilizer N, and increasing the NUE (Bahar et al., 2019).

Limitations to wide use in farms of SRFs and CRFs are determined by high product costs, low efficiency of early products and the use of petroleum-based synthetic polymers with uncertain environmental impact (Vejan et al., 2021). These types of fertilizers are much more expensive than classical fertilizers which makes their initial market smaller targeted to higher income crops, thus naturally the first applications have been in horticulture on small plots, and in hybrid seed maize production where income premiums allow for top tier technology usage. Unfortunately, the total use of SRFs and CRFs is much smaller than the total amount of fertilizers used globally (Bahar et al., 2019). Despite their continuous development, SRFs and CRFs still have few supporters in Europe, primarily due to their high prices (Wesołowska et al., 2021).

Maize (*Zea mays* L.) is the second most produced crop on Earth with a great flexibility of ecological growing environment. Giving its great importance, maize has been one of the main crops researched and improved genetically to unleash its production potential and to counter environmental challenges.

Hybrid maize seed since its introduction in large scale agriculture in 1920’s led to 3 times increase in yield compared to non-hybrid varieties (Duvick, 2005), due to heterozis effect, increased stress resistance, increased plant populations, and better use of fertilizers. Therefore, hybrid maize is rightly considered a big part of the green revolution as described by Norman Borlaug. The commercial production of hybrid maize seed requires technical information on agronomic management that allows obtaining the maximum yield of high-quality seed (Majeed et al., 2023). Therefore, maize seed is produced in the most technologically advanced farms requiring specific know-how and machines to obtain valuable hybrid seeds.

Fertilizers, especially nitrogen, are indispensable for achieving sufficient yields of maize, as new hybrids that can support high plant populations need high amounts of fertilizers. One of the biggest yield limiting factors after water in maize crops is fertilization, which needs to become more efficient to sustain high request of nutrients, at precise moments in time (Jeffrey and Gyles, 2003). Research has underlined that fertilization is essential for maize plant development with direct positive effect in plant height, stem thickness, foliage development, total leaf area and leaf area index, final dry matter development and yield (Law-Ogbomo and Law-Ogbomo, 2009). Maize requires high quantities of nitrogen (Minev et al., 2019), significantly increasing production costs (Zanão Júnior et al., 2019) and it must be underlined that the optimization of nitrogen fertilization in maize cannot be done on a global scale but must be done for specific soil and climatic conditions (Matev and Minev, 2020). Therefore, nitrogen use efficiency has been a subject of attention for a long time in agronomic science, due to its foremost influence on maize yield (Barșon et al., 2021).

The aim of the paper is to present the results of the performed research focused on analyzing and comparing the new types of fertilizers (slow-release and controlled-release fertilizers) to classical ones, in terms of yield (i), and phenological (ii) and phenotypical development of plants (iii) in hybrid seed maize production under irrigated conditions, as well as in researching the optimum time of application (iv) for each kind of the studied fertilizer.

MATERIALS AND METHODS

Research has been conducted in field experiments under irrigation conditions over three years (2020, 2021, and 2022) in the pedo-climatic conditions of the Moldavian plain, near Prut River in NE Romania (47.5188° N, 27.4490° E), in Bivolari commune, Iasi County. Research was performed in a farm specialized in hybrid seed production at maize and other field crops, respectively SC Integrasem S.R.L. which is producing hybrid seed of maize on a surface of 2,500 ha.

Field research was organized as subdivided experimentation plots with 4 replications and the following experimental factors:

- Factor A – nitrogen fertilizer product, with 5 treatments:
  - a1 = Control 1 – no added nitrogen fertilizer in spring;
  - a2 = Control 2 – no added nitrogen fertilizer in spring, but with complex fertilizers added at seedbed preparation;
  - a3 = ammonium nitrate added to Control 2, as classical nitrogen fertilizer (CNF);
  - a4 = Urea NG added to Control 2, as slow-release nitrogen fertilizer (SRNF);
  - a5 = Multicote (4) 34-0-7 added to Control 2, as control-release nitrogen fertilizer (CRNF).
- Factor B – fertilizer rate split between seedbed preparation and BBCH 16, respectively at growth stage of maize plants of 6 leaves unfolded, codified by BBCH (Meier, 2001), with 4 treatments:
  - b1 = 100% at seedbed preparation (SbP);
  - b2 = 50% at SbP + 50% at BBCH 16 side-dressing;
  - b3 = 25% at SbP + 75% at BBCH 16 side-dressing;
  - b4 = 100% at BBCH 16 side-dressing.

Control 1 variant received only 100 kg/ha of ammonium nitrate applied pre-ploughing in the autumn of the preceding year with the aim of sustaining the preceding crop residue decomposition. For this experimental variant, there was no fertilizer added in the spring.

Control 2 variant is the basic fertilization scheme used by the seed multiplier SC Integrasem S.R.L. and approved by the parent company of the inbred lines. Control 2 variant consisted of fertilization with 100 kg/ha of ammonium nitrate applied pre-ploughing in the autumn of the preceding year (as in the case of Control 1), plus 300 kg/ha of complex fertilizer $14:14:14+7SO_3+4MgO$ applied at seedbed preparation and 150 kg/ha of complex fertilizer 20:20:0+0.5Zn applied also at seedbed preparation. These fertilizers assured the following rates of nutrients: 105.5 kg/ha N; 72 kg/ha P_2O_5; 42 kg/ha K_2O; 21 kg/ha SO_3; 12 kg/ha MgO; 0.75 kg/ha Zn.

The nitrogen fertilizer products used in the performed research (ammonium nitrate, urea NG, and Multicote (4) 34-0-7) assured each a nitrogen rate of 67 kg/ha, this providing for all the experimental variants except Control 1 and Control 2 the same total nitrogen rate of 172.5 kg/ha (including in this amount the nitrogen applied at seedbed preparation through complex fertilizers).

Ammonium nitrate (33.5% N) is a classical fertilizer with rapid release of nitrogen. Urea NG (46% N) has its grains coated with a Neem extract (N-Guard Oil) film which assures a slow-release of nitrogen and consequently allows plants to absorb the nitrogen gradually over a longer period. Multicote (4) 34-0-7 (34% N and 7% K_2O) is a control-release fertilizer which is releasing nutrients over 4 months at soil temperature of 21°C.

The nitrogen application at BBCH 16 growth stage and side-dressing was chosen taking into account the maize has a sigmoidal nitrogen uptake pattern with a turning point at BBCH 16 from when nitrogen consumption increases exponentially. It is considered that, no matter of application method, the mineral nutrient content stored in maize leaves at BBCH 17 stage deeply impacts maize yields. The side-dressing application with cultivator was chosen having into account to cover fertilizer granules with soil for diminishing nitrogen losses through volatilization process (Gaj et al., 2012).

The size of the experimental variant was 192 m^2, which resulted from a width of 4.8 m, meaning 8 rows with 0.6 m spacing between rows, and a length of 40 m. Among the 8 rows, 6 were female rows and 2 were male rows.

The biological material used in the 3 consecutive years of research was the male and female parental lines of commercial hybrid P9889, a FAO 350 maize hybrid with medium precocity.

The seeding parity of the male and female parental lines was 6:2, with 6 female rows and 2 male rows with the row spacing of 60 cm and 18.3 cm between plants on row. The planting ratio of 6:2 is a newer ratio introduced in Romania in 2010's. The ratio of female to male rows of 6:2 is less complicated and less costly to handle with conventional planters and harvesters. It assures the increase of the surface per hectare of female harvestable plants (75%) compared to more classical ratios like 4:2 where only 67% of the surface is covered with female plants (Wright, 1980).

The planting schedule must consider the difference in maturity between the two parental lines together with the objective of having pollen in the field for a longer period. Thus, the first planting pass requires the planting of the female and first male (one male row), and the second male (the second male row) is to be planted after 33 GDD (Growing Degree Days with the biological threshold of 10°C). It is recommended to sow male rows on at least two consecutive dates, so that the pollination period of the plants on male rows completely encompasses the receptivity period of the plants on female rows (MacRobert et al., 2014).

In terms of tillage, it was applied conventional tillage with plowing at 27 cm in autumn, followed by a disc ripper to level the soil. In spring, the seedbed preparation was done at a depth of 6 cm in the day before sowing.

Sowing time was established by determination the temperature of 10°C at seeding depth for the first stage of planting Female + Male 1, while Male 2 was planted as stated after reaching 33 GDD. Practically, sowing was performed each year in the middle of April.

Weed control is essential in hybrid seed maize crops and thus a two-step herbicide program was implemented with Adengo 465 SC (225 g/l Isoxaflutole + 90 g/l Thiencarbazone-methyl + 150 g/l Cyprosulfamide - safener) 0.35 l/ha applied at BBCH 12 to control dicotyledonous and annual monocot weeds. Late post-emergence herbicide application of Laudis OD 66 (44 g/l Tembotrione + 22 g/l Isoxadifen-ethyl - safener) 2.25 l/ha at BBCH 18 has led to a field cleaning of weeds.

Irrigation consisted of 5 sprinkler irrigation applications of 30 mm: first irrigation was performed at BBCH 14 after herbicide application in order to activate it and support plant development; second irrigation was performed at BBCH 18 after second herbicide application and after fertilizer application in order to make the nutrients available as soon as possible; third irrigation was performed at BBCH 63, at tassel appearance to ensure water for the high consumption period at flowering; fourth irrigation was performed during flowering in order to sustain pollination and help plants surpass the high heat during that time; fifth irrigation was performed at grain filling time, which is critical to have good thousand grain weights results helping to obtain higher yields. Irrigation was applied with automated equipment with central pivot.

The crop technology was designed to assure optimum growing conditions for maize plants concerning nutrition, water supply and plant protection.

The soil on which the experiments were conducted is a very fertile one from the class of Chernozems. Ph is 7.52, total nitrogen content is 15.09 mg/kg, phosphorus content is 21.00 mg/kg, and potassium content is 228.54 kg/kg, according to soil analyses performed in 2022.

Under the Köppen climate classification the location of the study is within a warm-summer humid continental climate (Dfb). The multi-annual mean temperature is 11.16°C and multi-annual precipitations amount to 523.2 mm, from data collected for the past 61 years by the local meteorological station. Location altitude is 160 m above sea level.

Comparing the historical averages and the averages for 3 years of research, one can observe a consistent pattern of warming especially in the cold months between October and March with an average increase of 3°C for this period (Table 1). In terms of annual precipitation regime, the impact is more important with a decrease of 41.8 mm which shows deteriorating conditions for crop growing especially in the case of maize where the average decrease of 36 mm in July is occurring during the critical phase before flowering where maximum water availability is required (Table 2).

During the 3 years of research, the variability of conditions ensures that the field experiments were conducted under different meteorological conditions which increase the general character of the obtained results (Table 1 and Table 2).

Table 1. Monthly temperature and historical comparison

Year/Months	Temperature - Monthly Average (°C)												Annual Mean
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2020	1.1	4.1	7.2	11.2	14.6	21.9	22.3	23.5	19.6	14.4	5.3	2.4	12.3
2021	0.2	-1.6	3.7	8.6	15.4	20.9	24.1	21.3	15.7	9.9	6.6	0.0	10.4
2022	0.5	3.8	3.7	10.4	16.8	22.1	23.6	23.5	16.1	12.3	5.7	1.0	11.6
Average (A)	0.6	2.1	4.9	10.1	15.6	21.6	23.3	22.8	17.1	12.2	5.9	1.1	11.4
Historical Average (HA)	-3.8	-2.0	2.9	10.8	18.9	23.3	24.4	23.5	16.8	9.5	2.8	-1.1	10.5
Difference (A – HA)	4.4	4.1	2.0	-0.7	-3.3	-1.7	-1.1	-0.7	0.3	2.7	3.1	2.2	0.9

Table 2. Precipitation regime and historical comparison

Year/Months	Rainfall - Monthly Average (mm)												Annual Sum
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2020	3.4	32.0	15.7	1.8	132.0	98.4	30.4	5.8	26.3	80.1	13.0	41.0	479.9
2021	19.1	28.2	45.4	40.2	67.4	109.4	40.5	132.8	6.6	3.5	10.3	61.2	564.6
2022	9.1	10.8	8.4	52.7	21.5	35.1	28.4	61.3	85.6	13.5	54.4	18.9	399.7
Average (A)	10.5	23.7	23.2	31.6	73.6	81.0	33.1	66.6	39.5	32.4	25.9	40.4	481.4
Historical Average (HA)	27.9	25.8	28.4	43.4	55.1	80.7	69.1	55.4	52.3	23.5	30.7	30.9	523.2
Difference (A – HA)	-17.4	-2.1	-5.2	-11.8	18.5	0.3	-36.0	11.2	-12.8	8.9	-4.8	9.5	-41.8

The year 2020 registered the highest recorded annual average of 12.3°C and high temperatures in July and August, to which one adds the effect of pedological draught starting in the second decade of July and the whole month of August, when total precipitations amounted 5.8 mm. This climate context impacted heavily on yield establishment as there were improper conditions at maize flowering time and on pollen viability.

The year 2021 was a good year to grow maize in NE Romania as temperatures were cooler than historical averages across the growing period which, together with 41.4 mm of extra yearly precipitation mostly in June and August, facilitated exceptional conditions at pollination time especially.

The year 2022 was the worst climatic year for maize growing in the past 60 years. Rainfall had a gross deficit of 123.5 mm with monthly averages less than 50% of the historical averages in May, June, and July which gravely impacted crop establishment and yield formation.

Phenological determinations aimed at the important dates for crop development in the case of maize for producing hybrid seeds. For crop establishment there were noted the planting date of plants on female rows and male 1 and male 2 rows and the emergence date of each inbred line. For flowering time there were noted the date when 10% of female plants were at silking stage (with stigmata visible), as well as the date when 10% of male plants on the two rows (male 1 and male 2) had tassel emerged. Also, it was noted the detasseling date and end of flowering time for each inbred line, as well as the date of harvest.

Phenotypical determinations aimed the plant development in terms of plant height and number of leaves before detaselling.

Cob harvest was done manually at grain humidity of 34%, which is ideal for cob harvesting. A number of 400 cobs were harvested per variant, i.e. 4 repetitions x 100 cobs. The grain yield was determined by shelling the grains from the cobs. Also, the yield components of the cob were determined as well as the thousand grain weight.

To determine the statistical significance of the set of primary data, obtained in the field experiments, it was used the test package from the Microsoft Excel statistical analysis extension XLSTAT. Further analysis was conducted using Fisher test to strengthen the analysis of differences between variants and control (P-value < 0.05). Dunnett test was used to compare variant by variant with the control. Tukey test was used to test for significance of all pairwise comparisons, which compares each variant against each other and against control variants to find the most statistically significant variants which are the best options to propose as recommendations for use in practice.

RESULTS AND DISCUSSIONS

Analysis of the average yield results of the 3 years of the performed research, shown in Table 3, has put into evidence the variant fertilized with slow-release Urea NG with 100% rate (67 kg/ha of nitrogen) applied at seedbed preparation, in this variant being obtained the highest average yield of 10,017.5 kg/ha, which means an increase of 47.21% compared to Control 1 variant and an increase of 20.81% compared to Control 2 variant. At the same time, 3 out of the top five yielding variants have used Urea NG as fertilizer, which highlight slow-release fertilizers efficiency in providing sufficient nutrition over the growth period of the hybrid seed maize crop and the correlation between the release of nutrients and consumption curve. Close to the best variant with slow-release fertilizer Urea NG was the variant with the control-released fertilizer Multicote (4) 34-0-7 with 50% of the rate applied at seedbed preparation and 50% of the rate applied at growth stage of BBCH 16. These two best fertilization variants registered the smallest coefficient of variation in the three experimental years, respectively 0.86% and 1.00% (Table 3). This is explained by the long nitrogen release of these fertilizers which makes the nitrogen available to be assimilated by maize plants over the whole growth period, regardless of water availability.

Ammonium nitrate variants with shorter release periods of active substance are susceptible to leaching if there is too much water due to precipitation and can cover only a part in the growing period of the plants not well correlated with their need but more with water availability in the soil. So, the best ammonium nitrate variant was the one with 100% of the rate applied at growth stage of BBCH 16 (9899.2 kg/ha), but with a high coefficient of variation (8.73%), which shows the dependence on the climatic conditions of the year. The ammonium nitrate variant with 100% of the rate applied at seedbed preparation registered the smallest yield (9467.5 kg/ha) among all the experimental variants, with a small coefficient of variation (2.84%), which shows the constancy of this variant regardless the climatic conditions of the year.

Yield performance by type of product highlights a higher average yield for slow-release fertilizer Urea NG (9840 kg/ha) compared to controlled release fertilizer Multicote (4) 34-0-7 (9721 kg/ha) followed by Ammonium Nitrate (9696 kg/ha) (Figure 1). Control variants have been clearly outperformed by all the variants with nitrogen fertilizer.

Table 3. Hybrid seed yield ranking of variants

Experimental variant	Hybrid seed yield (2020-2022) Average (kg/ha)	Difference (%) compared to Control 1	Difference (%) compared to Control 2	Significance	Coefficient of Variation for 2020-2022 (%)
Urea NG – 100% SbP	10017.5	47.21%	20.81%	A	0.86%
Multicote (4) 34-0-7 – 50% SbP + 50% BBCH 16	9961.7	46.39%	20.14%	A	1.00%
Ammonium Nitrate – 100% BBCH 16	9899.2	45.32%	19.39%	A	8.73%
Urea NG – 50% SbP + 50% BBCH 16	9886.7	45.29%	19.24%	AB	6.00%
Urea NG – 100% BBCH 16	9767.5	43.53%	17.80%	AB	3.82%
Multicote (4) 34-0-7 – 100% BBCH 16	9750.8	43.29%	17.60%	AB	6.44%
Ammonium Nitrate – 50% SbP + 50% BBCH 16	9749.2	43.27%	17.58%	AB	1.13%
Urea NG – 25% Seedbed + 75% BBCH 16	9686.7	42.35%	16.82%	AB	8.05%
Ammonium Nitrate – 25% SeP + 75% BBCH 16	9680.0	42.25%	16.74%	AB	9.29%
Multicote (4) 34-0-7 – 25% SbP + 75% BBCH 16	9680.0	42.25%	16.74%	AB	6.44%
Multicote (4) 34-0-7 – 100% SbP	9492.5	39.49%	14.48%	AB	5.35%
Ammonium Nitrate – 100% SbP	9467.5	39.13%	14.18%	AB	2.84%
Control 2 – no nitrogen fertilizer in spring, but complex fertilizers applied at seedbed	8291.7	21.85%	-	BC	2.95%
Control 1 – no nitrogen fertilizer in spring	6805.0	-	-17.93%	C	3.65%

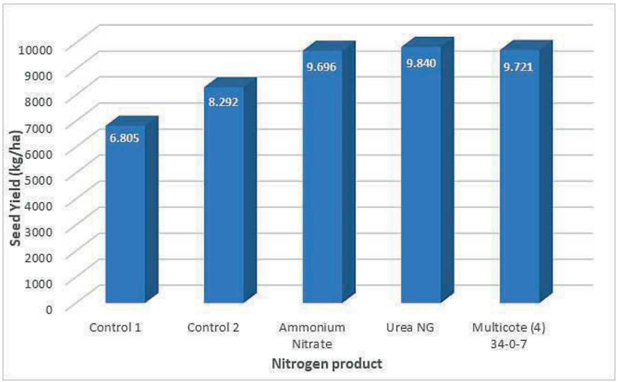

Figure 1. Average yield by specific nitrogen fertilizer type

The yield distribution of the variants shown in Figure 2 illustrates 2 clusters of yields one around the value of 9,000 kg/ha slightly above the values of the control 2 variant which varied around 8,300 kg/ha. The second main cluster around the value of 9,500 kg/ha consistent mostly of slow-release fertilizer at the top of the cluster above 10,000 kg/ha and controlled release fertilizer Multicote 34-0-7 in the upper echelon of the second cluster at around 9,600 kg/ha. It can be highlighted the fact that even though not all the variants with controlled release and slow-release fertilizers are highest performers, they are all situated in the top quartiles of yielding variants.

Statistical analysis of yield results of show that the type of fertilizer has a great importance in explaining the yields for each variant as R^2 coefficient has a high value of 0.715 (Table 4) which shows the strong relationship between fertilizer type and yield. Fisher test results $P<0.0001$ (Table 5) further strengthen the previous observation as it is a test that can show the correlation between factors and yield

even in conditions of small statistical sample sizes. The performed study is therefore statistically confirmed as being an analysis of a critical element that greatly influences final yields and the yields of slow-release and controlled-release fertilizer variants are the highest.
Using the statistical analysis tool Tuckey Test, that allows a comparison between variants and comparison to control variants, one can confidently observe that there are statistically significant differences between 4 tested variants and the rest of tested variants (Table 6).
The highest yielding variant (10,017.5 kg/ha) was Urea NG with the entire rate applied at seedbed preparation (Figure 3), which is consistent with its release pattern over 4 months which covers high N consumption stages especially between BBCH 16 and flowering. This highlights the good slow-release mechanism of this product which is correlated with maize nitrogen consumption curve. An alternative option when using Urea NG is splitting the application to 50% at seedbed preparation and 50% at BBCH 18. But, even though significant increases in yield can be observed in the case of 50/50 application of Urea NG, this option had a lower yield that classical, cheaper, fertilizer ammonium nitrate applied 100% at BBCH 16 growing stage, and the farmer will lose the economic benefit of having one less pass in the field.

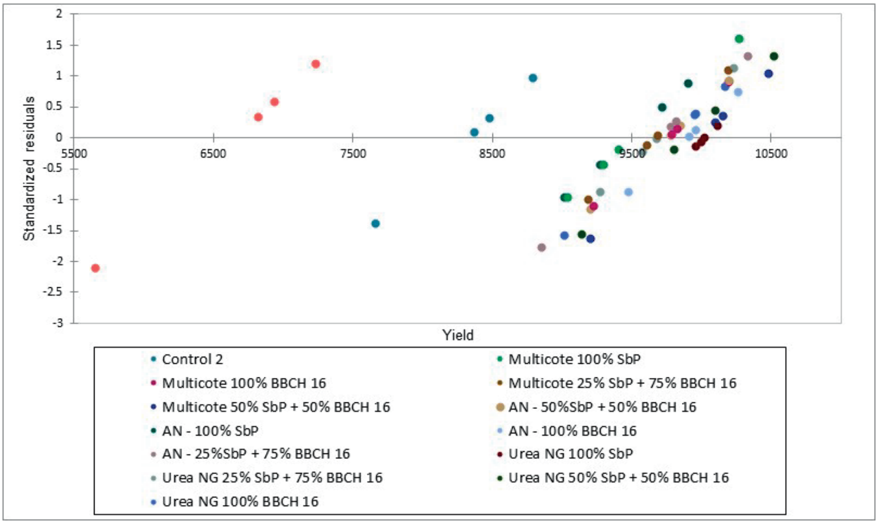

Figure 2. Yield distribution of variants

Table 4. Goodness of fit R^2

Observations	56
Sum of weights	56
DF	42
R^2	0.715
Adjusted R^2	0.627

Table 5. Fisher test values

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	13	39027419.246	3002109.173	8.103	**<0.0001**
Error	42	15561313.889	370507.474		
Corrected Total	55	54588733.135			

Table 6. Tukey test multi comparison test for hybrid seed yields according to moment of fertilizer application

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Urea NG – 100% SbP	10017.500	304.347	9403.304	10631.696	A		
Multicote (4) 34-0-7 – 50% SbP + 50% BBCH 16	9961.700	304.347	9347.470	10575.863	A		
Ammonium Nitrate – 100% BBCH 16	9899.200	304.347	9284.970	10513.363	A		
Urea NG – 50% SbP + 50% BBCH 16	9886.700	304.347	9272.470	10500.863	A		
Urea NG – 100% BBCH 16	9767.500	304.347	9153.304	10381.696	A	B	
Ammonium Nitrate – 50% SbP + 50% BBCH 16	9750.800	304.347	9136.637	10365.030	A	B	
Urea NG – 25% SbP + 75% BBCH 16	9749.200	304.347	9134.970	10363.363	A	B	
Multicote (4) 34-0-7 – 100% BBCH 16	9686.700	304.347	9072.480	10300.863	A	B	
Multicote (4) 34-0-7 – 25% SbP + 75% BBCH 16	9680.000	304.347	9065.804	10294.196	A	B	
Ammonium Nitrate – 25% SbP + 75% BBCH 16	9680.000	304.347	9065.804	10294.196	A	B	
Multicote (4) 34-0-7 – 100% SbP	9492.500	304.347	8878.304	10106.696	A	B	
Ammonium Nitrate – 100% SbP	9467.500	304.347	8853.304	10081.696	A	B	
Control 2	8291.700	304.347	7677.470	8905.863		B	C
Control 1	6805.000	304.347	6190.804	7419.196			C

Controlled release fertilizer Multicote (4) 34-0-7 was the second-best option with 9961 kg/ha with a split application 50% at seedbed preparation and 50% at BBCH 16 side-dressed which is optimal with the slower release of controlled release fertilizers through hydrophobic polymers compared to neem oil treated Urea NG (Figure 3).

Yield distribution for ammonium nitrate by split rate has clearly shown that the use of ammonium nitrate generates the highest yield when applied the integral rate at BBCH 16 (Figure 3). This confirms the fact that the pattern of nitrogen release for ammonium nitrate granules follows a curve with initial exponential growth which slows down after a month, thus ammonium nitrate is the optimal solution to be applied before the maximum growth intensity period from BBCH 16 to BBCH 61.

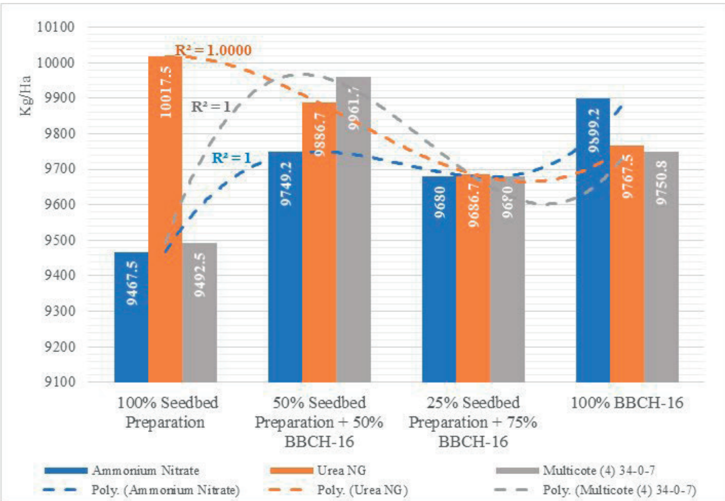

Figure 3. Average yield according to nitrogen fertilizer and moment of application

Controlled release fertilizers are best applied in 50-50 split between seedbed preparation and BBCH 16 side dressed, and slow-release fertilizers are best applied the entire rate at seedbed preparation. The use of slow release and controlled release fertilizers at the right moment of application will allow the reduction of physical quantities of fertilizer applied per hectare for the same nitrogen rate by 27% if using Urea NG and 1.5% if using Multicote 34-0-7 compared to ammonium nitrate fertilizer.

The Dunnett test (Table 7) which compares each variant with the control shows that the variant with ammonium nitrate with the entire rate applied at seedbed preparation and the one with Multicote controlled release fertilizer also applied the entire rate at seedbed preparation have not exhibited statistically significant differences compared to the control. This statistically significant discovery is consistent with the release curve of the two types of fertilizer. Thus, ammonium nitrate with nitrate and ammonium nitrogen is applied too early and is prone to leaching, the performed research being under irrigation condition, and will not be available for later stage development of maize plants. Concerning Multicote fertilizer, the hydrophobic coating will lead to a very slow release of nitrogen in initial stages of vegetation which will lead to poor crop establishment which plagues through all the future growth stages.

Phenotypical determinations conducted over the growth cycle for each of the 3 years have produced results that sustain the findings regarding yields. The variants that exhibited significant statistical differences compared to all other variants such as Urea NG with the entire rate applied at seedbed preparation, Multicote 50-50 split and Ammonium Nitrate with the entire rate applied at BBCH 16, all have resulted in exceptional phenotypical development with a plant height of over 200 cm for female inbreds before detasseling and at the same time a very good development of foliar apparatus (Table 8).

Table 7. Dunnett test control comparison test

Contrast*	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
C2 vs Urea NG – 100% SbP	-1725.833	-4.010	2.920	1256.610	0.003	Yes
C 2 vs Multicote (4) 34-0-7 – 50% SbP + 50% BBCH 16	-1670.000	-3.880	2.920	1256.610	0.004	Yes
C2 vs Ammonium Nitrate – 100% BBCH 16	-1607.500	-3.735	2.920	1256.610	0.006	Yes
C2 vs Urea NG – 50% SbP + 50% BBCH 16	-1595.000	-3.706	2.920	1256.610	0.006	Yes
C2 vs Urea NG – 100% BBCH 16	-1475.833	-3.429	2.920	1256.610	0.014	Yes
C2 vs Multicote (4) 34-0-7 – 100% BBCH 16	-1459.167	-3.390	2.920	1256.610	0.015	Yes
C2 vs Ammonium Nitrate – 50% SbP + 50% BBCH 16	-1457.500	-3.386	2.920	1256.610	0.015	Yes
C2 vs Urea NG – 25% SbP + 75% BBCH 16	-1395.000	-3.241	2.920	1256.610	0.022	Yes
C2 vs Ammonium Nitrate – 25% SbP + 75% BBCH 16	-1388.333	-3.226	2.920	1256.610	0.023	Yes
C2 vs Multicote (4) 34-0-7 – 25% SbP + 75% BBCH 16	-1388.333	-3.226	2.920	1256.610	0.023	Yes
C2 vs Multicote (4) 34-0-7 – 100% SbP	-1200.833	-2.790	2.920	1256.610	0.068	No
C2 vs Ammonium Nitrate – 100% SbP	-1175.833	-2.732	2.920	1256.610	0.078	No
C2 vs C1	1486.667	3.454	2.920	1256.610	0.013	Yes

*SbP = Seedbed preparation; C1 – Control 1; C2 – Control 2

Table 8. Maize plant height and number of leaves per plant at detasseling according to nitrogen fertilizer and moment of application

Nitrogen fertilizer		Plant height at detasseling (cm)					Number of leaves at detasseling				
Fertilizer product	Fertilizer rate split (kg/ha)	2020	2021	2022	Average	Significance	2020	2021	2022	Average	Significance
Control 1	–	179	185	173	179	C	13	15	12	13	D
Control 2	–	181	188	176	182	BC	15	16	14	15	C
Ammonium nitrate	100% SbP	184	192	177	184	B	15	16	15	15	C
	50% SbP + 50% BBCH 16	189	197	179	188	B	16	17	14	16	BC
	25% SbP + 75% BBCH 16	186	195	178	186	B	16	17	15	16	BC
	100% BBCH 16	200	207	191	200	A	17	18	15	17	AB
Urea NG	100% SbP	206	213	195	205	A	18	19	16	18	A
	50% SbP + 50% BBCH 16	198	206	190	198	A	17	18	16	17	B
	25% SbP + 75% BBCH 16	196	203	189	196	AB	16	18	15	16	BC
	100% BBCH 16	187	195	180	187	AB	16	17	15	16	BC
Multicote (4) 34-0-7	100% SbP	186	196	177	186	AB	15	18	14	16	BC
	50% SbP + 50% BBCH 16	203	210	193	203	A	17	18	15	17	AB
	25% SbP + 75% BBCH 16	194	200	186	193	AB	15	18	15	16	BC
	100% BBCH 16	195	203	188	195	AB	17	18	16	17	B

Urea NG applied pre-planting (at seedbed preparation) resulted in the highest number of leaves (18 leaves) followed by ammonium nitrate applied at BBCH 16 and Multicote 50-50 split. Thus, slow-release fertilizers with the entire rate applied at seedbed preparation, controlled release fertilizers applied 50% at seedbed preparation and 50% at BBCH 16, and classic fertilizers with the entire rate applied at BBCH 16 are the best options for optimum phenotypical development of hybrid maize seed crops.

Maize is a monoecious plant with a tendency towards protandrous development, especially under high stress conditions such as draught and dry air, thus the anthers of the male tassel open about 3 days before the emergence of female stigmata.

Field evaluations show that the average time between flowering of male 1 tassel and female stigmata emergence was 4 days for most of the variants. The 4 variants that proved to be superior in terms of yield were also superior in terms of flowering gap (Table 9). Urea NG applied at seedbed or 50/50 split between seedbed and BBCH 16, Multicote applied in a 50/50 split, together with ammonium nitrate applied at BBCH 16 had an optimum flowering gap of 3 days between male and female inbreds.

Irrigation performed in all the three experimental years during flowering sustained pollination and helped plants surpass the high heat during that time. This is important knowing that high temperatures in this phase increase the gap between the appearance of tassel and that of silks, and at a temperature of 28-30°C, the viability of the pollen decreases (Mircea et al., 2021).

The number of grains per cob depends on the type of nitrogen fertilizer (Szulc et al., 2023). The optimum flowering gap leads on average to an extra grain per cob as better pollination was realized due to the extra day of pollen availability. This extra grain as a yield formation element had an impact on final yield as exhibited by data presented.

Table 9. Flowering gap and number of grains per row according to nitrogen fertilizer type

Nitrogen fertilizer		Flowering Gap (days)					Number of grains per cob				
Fertilizer product	Fertilizer rate split	2020	2021	2022	Average	Significance	2020	2021	2022	Average	Significance
Control 1	–	5	4	6	5	C	195	223	212	210	C
Control 2	–	4	3	4	4	B	217	238	217	224	BC
Ammonium nitrate	100% SbP	4	4	5	4	B	224	231	217	224	BC
	50% SbP + 50% BBCH 16	4	4	5	4	B	224	273	217	238	AB
	25% SbP + 75% BBCH 16	4	4	5	4	B	224	273	217	238	AB
	100% BBCH 16	3	3	4	3	A	221	311	224	252	A
Urea NG	100% SbP	3	3	4	3	A	231	302	223	252	A
	50% SbP + 50% BBCH 16	3	3	4	3	A	223	310	223	252	A
	25% SbP + 75% BBCH 16	4	3	5	4	B	231	259	224	238	AB
	100% BBCH 16	4	4	5	4	B	224	231	224	238	AB
Multicote (4) 34-0-7	100% SbP	4	4	5	4	B	223	273	218	238	AB
	50% SbP + 50% BBCH 16	3	3	4	3	A	231	302	223	252	A
	25% SbP + 75% BBCH 16	4	3	4	4	B	223	274	217	238	AB
	100% BBCH 16	4	3	4	4	B	223	274	217	238	AB

The finding that the use of Urea NG used at seedbed preparation is the most productive option is in connection with a study of Zhang et al. (2019) who highlighted that the use of controlled release urea has resulted in an increase of maize yield by 5.3% as well as increased nitrogen use efficiency by 24.1%, and at the same time it meant a reduction in total nitrous oxide (N_2O) emission, N leaching as well as ammonia (NH_3) volatilization by 23.8%, 27.1% and 39.4%, respectively.

The results showing ammonium nitrate applied through side-dressing at BBCH 16 stage as a significant yield increasing variant are consistent with those obtained by Bășa et al. (2016) saying that nitrogen application in the growth stage of five leaves of the maize plants increased the values of the yield component and grain yield.

The findings regarding split application of controlled release fertilizer and pre-plant application of slow-release fertilizers are according to those of Arrobas et al. (2022) who found that for the same N rates, CRF and SF (stabilized fertilizers) gave similar DM yields, not dissimilar or even higher plant N nutritional status indices and N use efficiencies, than the conventional fertilizer split into two applications.

The finding of enhanced phenotypical development of variants fertilized with slow-release fertilizers can be corroborated with the findings of Li et al. (2020) where over ground parts dry weight has increased by 10.52-17.49%, for slow-release fertilizers, this being consistent with superior phenotypical development of variants where they have been used.

Crucial for consistent yields in hybrid seed maize production is achieving a synchronization called pollination nicking (Wright, 1980), between male anthers pollen shedding and emergence of female stigmata from husks, to have the highest rate of pollination of a kernel. The period between male and female flowering is vital because pollination, fertilization, and eventually grain filling and grain yield depend on it (Sirih et al., 2021). It is necessary to monitor the accumulated GDD to detect on time temperature changes that may modify days to flowering, the detasseling schedule, as well as the grain-filling period duration, and therefore effective seed yield improvement (Sirih et al., 2021). Therefore, an essential element to be observed when considering technological changes is their effect on nicking. For hybrid seed maize crops it is important that the carefully sought after flowering synchronization between female and male inbred is not perturbed by any of the changes made to fertilization schemes.

The length of pollen release period observed in our study is confirmed with that of Chassaigne-Ricciulli et al. (2021) who found that both the mean period of pollen release of the male inbred lines as well as the period of exposure of stigmas of the female single crosses were 4 to 5 days.

The better development of yielding elements in variants with slow release urea, applied at seedbed preparation, and controlled release fertilizer, evenly split between seedbed preparation and BBCH 16, is consistent with Li et al. (2020) which found that compared with common urea, grain number per ear, 1000-grain weight and yield of maize of the treatments with controlled-release urea were overall significantly increased, among which the yield was significantly increased by 14.81-25.84%.

It has to be considered the economic benefit of reducing one cultivation pass in the case of variants with all fertilizer applied at seedbed preparation or in vegetation period which will bring a decrease in cost per hectare (20 Euro/ha on average in the conditions of the performed research). At the same time a reduction of the number of passes on the soil will have a positive impact on soil compaction.

Logistic costs are greatly reduced as if one uses the highest yielding variant in practice, such as Urea NG all applied at seedbed preparation, which need 30% less commercial product in weight, and 1.5% less than the use of controlled release Multicote 34-0-7. As the performed study showed, the use of slow-release fertilizers can be applied as a pre-planting, production costs can be reduced, eliminating the need for multiple applications of soluble nitrogen fertilizers (Abd El-Azeiz et al., 2021).

CONCLUSIONS

The highest average hybrid grain yield at maize (10,017 kg/ha) were obtained in the case of variant fertilized with slow-release Urea NG with the whole rate (67 kg nitrogen/ha) applied at seedbed preparation and in the case of variant fertilized with control-released fertilizer Multicote (4) 34-0-7 50-50 split between seedbed preparation and BBCH 16 side dressed (9961 kg/ha).

The obtained results sustain the use of slow release and controlled release fertilizers in hybrid seed maize production, which will lead to better yields compared to classical fertilization options, no negative impact on synchronization at pollination time, lower quantities of physical fertilizer product to be used, and reduced number of passes in the field. Practically, all these make the use of this type of fertilizers desirable in hybrid seed maize production.

The best moment of application of nitrogen fertilizers in maize seed production differs according to fertilizer type. Urea NG or other similar slow-release fertilizers which use neem extract as a nitrification inhibitor are best applied at seedbed preparation. Controlled release fertilizers such as Multicote 34-0-7 are best applied 50% at seedbed preparation and 50% at BBCH 16 with cultivation. Ammonium nitrate should be applied in the vegetation period (BBCH 16) with cultivation.

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