ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2021; 26(6): 3129-3136
doi: 10.25083/rbl/26.6/3129-3136

Received for publication, November, 18, 2021
Accepted, December, 12, 2021

*Original paper*

# *Yield results obtained in maize crop following the foliar fertilization with new biostimulators based on keratin and collagen hydrolysates of animal origin*

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**Abstract** The aim of the paper is to show the reaction of maize crop to the foliar application of new biofertilizers, obtained from the residues left after the wool tanning process and which are based on protein hydrolysates of keratin and collagen. The tests have been performed in microplots (10 sqm) and macroplots (1000 sqm), in three repetitions, in southern Romania. All data obtained as a result of the 2020 autumn harvest have been centralized in tables and statistically processed, in order to establish the influence of each product, compared to the control. The bifactorial analyses show that, in climatic stress conditions (prolonged drought – Calarasi), very significant increases in yield have been obtained with K4 (2.5 and 5.0 l/ha), K5 (5.0 l/ha) and C (5.0 l/ha), with increases between 700 and 1300 kg/ha, ie up to +24%. In macroplots, subject to a moderate drought (Teleorman), product C (5.0 l/ha) brought a yield increase of 1537 kg/ha (+26%), noting that in this location almost all tested biostimulators offer very significant yield growth (except KC). Based on the 2020 yields, it was possible to identify which the foliar biostimulators that will continue to be tested in 2021.

**Keywords** maize, foliar fertilization, biostimulator, keratin hydrolysate, yield

**To cite this article:** BECHERIȚU M, HOROIAȘ R, CIOINEAG C, BOROVINĂ P, GAIDĂU C, NICULESCU M. Yield results obtained in maize crop following the foliar fertilization with new biostimulators based on keratin and collagen hydrolysates of animal origin. *Rom Biotechnol Lett*. 2021; 26(6): 3129-3136. DOI: 10.25083/rbl/26.6/3129-3136.

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# Introduction

It has been repeatedly proven that foliar fertilization can compensate an insufficient nutrition from the soil, as long as the foliar surface is well developed (LING & SILBERBUSH, 2002 [1]; FERNANDEZ & EICHERT, 2009 [2]; BRANKOV et al, 2020 [3]), and the amount of fertilizer is well correlated with the needs of the plants (GIRMA et al, 2007 [4]; ANDRIC et al, 2016 [5]; BERNHARD, 2016 [6]; ADAMEC et al, 2020 [7]).

In addition, for foliar fertilization, products of natural origin can be used with much higher efficiency (FERNANDEZ et al, 2013 [8]), to replace some of the synthetic nutrients (MONDA et al, 2018 [9]), with long-term economic and ecological effects (FRANCIS et al, 2016 [10]). Biostimulators are that kind of products, having the role of promoting the growth, the development and the productivity of plants (KOCIRA et al, 2020 [11]).

In maize crop, it has been shown that biostimulators improve the absorption of nutrients by plants, especially by increasing the leaf (DA SILVA, 2018 [12]) and roots (TADROS et al, 2019 [13]) biomass, but also by accelerating the transport or translocation of nutrients and the enzymatic activity involved in their assimilation (BULGARI et al, 2019 [14]). At the same time, the role of biostimulators is to increase the tolerance level of plants against abiotic stresses (HU et al, 2008 [15]; BROWN & SAA, 2015 [16]; TREVISAN et al, 2019 [17]; VAN OOSTEN et al, 2017 [18]), which can be quantified by the way plants evolve during vegetative cycles (HOROIAS et al, 2021 [19]), and then by quantitative and qualitative evaluation of the obtained yields (JAKAB-GABOR et al, 2017 [20]).

The use of animal waste in agriculture is a process increasingly tested in recent years, used in different crops (ERTANI et al, 2013 [21]; BECHERITU et al, 2020 [22]). Obtaining efficient biostimulators with minimal costs, from the residues left as a result of wool processing (BEN et al, 2020 [23]; GAIDAU et al, 2021 [24]), would be an important achievement for producers, but also for farmers (YAKHIN et al, 2017 [25]).

# Materials and Methods

***Choosing the biological material – maize hybrid***

As a first step, the biological material that is going to be used in order to test the new biostimulators on the maize crop has been chosen. Taking into consideration the research area (southern Romania) pedoclimatic conditions, the Olt hybrid (a Romanian one, included in the 430 FAO group) has been considered to be the most suitable. The selection criterion was also based on continuity, knowing that this hybrid it will be available for the next two years of research.

***Establishing the locations for field research***

Choosing locations for the testing plots has been based, mostly, on the rainfall regime from the beginning of 2020, respectively January-April, an extremely useful information in order to know what is the water resource from the soil. All data were collected in Table 1, subsequently supplemented with data up to the end of August 2020, thus having the precipitation balance for the entire maize growing period.

**Table 1.** Precipitation recorder in the two locations where the biostimulators field tests were conducted – micro- and macroplots

| Crt. | Month | Precipitation (mm) | |
|---|---|---|---|
| | | **Modelu (Calarasi) - microplots** | **Calomfiresti (Teleorman) - macroplots** |
| 1. | January | 2 | 6 |
| 2. | February | 31 | 68 |
| 3. | March | 16 | 74 |
| 4. | April | 11 | 20 |
| ***5.*** | ***Total*** | ***60*** | ***168*** |
| 6. | May | 75 | 61 |
| 7. | June | 105 | 105 |
| 8. | July | 18 | 17 |
| 9. | August | 3 | 22 |
| **10.** | **TOTAL** | **261** | **373** |

The elements that determined us to carry out field tests in these two locations were the different distribution of monthly precipitation, as well as the fact that, until the time of sowing (April 20, 2020), in Teleorman county the soil had stored a volume of water 108 mm higher than in Calarasi county, on the background of a more balanced distribution. For the rest of the period (May-August 2020), the rainfall regime of the two locations was similar.

Regarding the pedological conditions of the two locations, these are similar, both falling into the forest-steppe category, with chernozems rich in clay (about 30%) and an amount of humus of about 3%.

***Agrotechnics of the testing plots***

The two research fields (from Calarasi and Teleorman counties) benefited from an identical agrotechnics. Both were placed after the winter wheat. The incorporation of the vegetal remains was done in September 2019, at the same time with the soil preparation for autumn sowing.

In April 2020, at the germination bed preparation for the spring crops, an amount of 250 kg/ha NPK (16:16:16) was incorporated. Fertilization was supplemented with an amount of 150 kg/ha ammonium nitrate, applied at sowing time, together with the seed. Basic fertilization was also applied to control variants.

Subsequently, the two foliar fertilizations with biostimulators (in the stages of 3-4 and 7-8 leaves) were performed. Along with the first foliar fertilization, herbicide was applied.

***Biostimulators created fort the foliar fertilization***

The nine biostimulators proposed by the tanning institutes from Romania (the first seven) and Czech Republic (the last two) show the characteristics presented in detail in Table 2. All keratin and collagen hydrolysates were adjusted to a neutral pH (approximately 7) in order not to be harmful to the plants.

**Table 2.** Presentation of biostimulators under test – main features

| No. | Biostimulator | Characteristics |
|---|---|---|
| 1. | **K1** (keratin hydrolysate) | - obtained with Protamex (1 g/l);<br>- dry substance = 10.60%;<br>- total N = 12.64%;<br>- protein substance = 76.60%. |
| 2. | **K2** (keratin hydrolysate) | - obtained with Esperase (1 g/l);<br>- dry substance = 9.26%;<br>- total N = 13.07%;<br>- protein substance = 79.20%. |
| 3. | **K3** (keratin hydrolysate) | - obtained with Valkerase (1 g/l);<br>- dry substance = 9.24%;<br>- total N = 12.12%;<br>- protein substance = 73.45%. |
| 4. | **K4** (keratin hydrolysate) | - dry substance = 7.87%;<br>- total N = 12.71%;<br>- protein substance = 77.02%. |
| 5. | **K5** (keratin hydrolysate) | - dry substance = 7.79%;<br>- total N = 12.58%;<br>- protein substance = 76.23%. |
| 6. | **KC** (keratin hydrolysate + collagen hydrolysate + macro- + microelements) | - dry substance = 14.87%;<br>- total N = 8.00%;<br>- protein substance = 47.69%. |
| 7. | **C** (collagen hydrolysate) | - dry substance = 9.63%;<br>- total N = 13.08%;<br>- protein substance = 73.51%. |
| 8. | **FM1** (collagen hydrolysate) | - dry substance = 28.88%;<br>- total N = 14.65%;<br>- protein substance = 88.78%. |
| 9. | **FM2** (collagen hydrolysate) | - dry substance = 29.29%;<br>- total N = 14.17%;<br>- protein substance = 85.87%. |

Following the analysis of the foliar fertilizer market, aiming to obtain an efficient product, but also affordable for farmers from the economical point of view, the quantities of 2.5 and 5.0 l/ha have been projected to be tested, with dilution in 300-500 l water.

***Drawing up the technological scheme***

In order to be able to draw conclusions with great accuracy, for both locations and types of experiments (micro- and macroplots) the use of the same technological scheme was chosen (Figure 1), only the dimensions of the plots being different. Each experimental field has been arranged in randomized blocks and three repetitions were placed.

Eventually, two experimental fields have been designed, with the following surfaces:

1) microplots (Calarasi) – 1500 sqm sown, out of which 1199 sqm were actually used, including the space of one meter between plots;

2) macroplots (Teleorman) – 7 ha sown, out of which 6,36 ha were actually used, including the space of one meter between plots.

The differences between the sown areas and those actually used are represented by the protection strips on the sides of the research lots.

***Performing laboratory analyses and data calculation***

Harvesting was carried out very carefully, each plot at a time. For accuracy, in microplots the harvesting was done manually, and in macroplots it was mechanized, the machine being very well cleaned after each plot.

Subsequently, samples from each plot were analyzed in the laboratory, using the Infratec Foss device (Figure 2), obtaining data on moisture (%), protein (%), hardness (%), oil content (%).

Knowing the yield per plot (kg of grains) and the harvest moisture (%), it was possible to calculate the actual yield (kg/ha). The last parameter measured was the weight of 1000 grains (WTG, g).

All data were entered in tables, and then statistically processed, by categories, focusing on yields, which most clearly highlighted the effects of foliar fertilization with the tested biostimulants. Graphs and significance were obtained by processing the data with the Anova program.

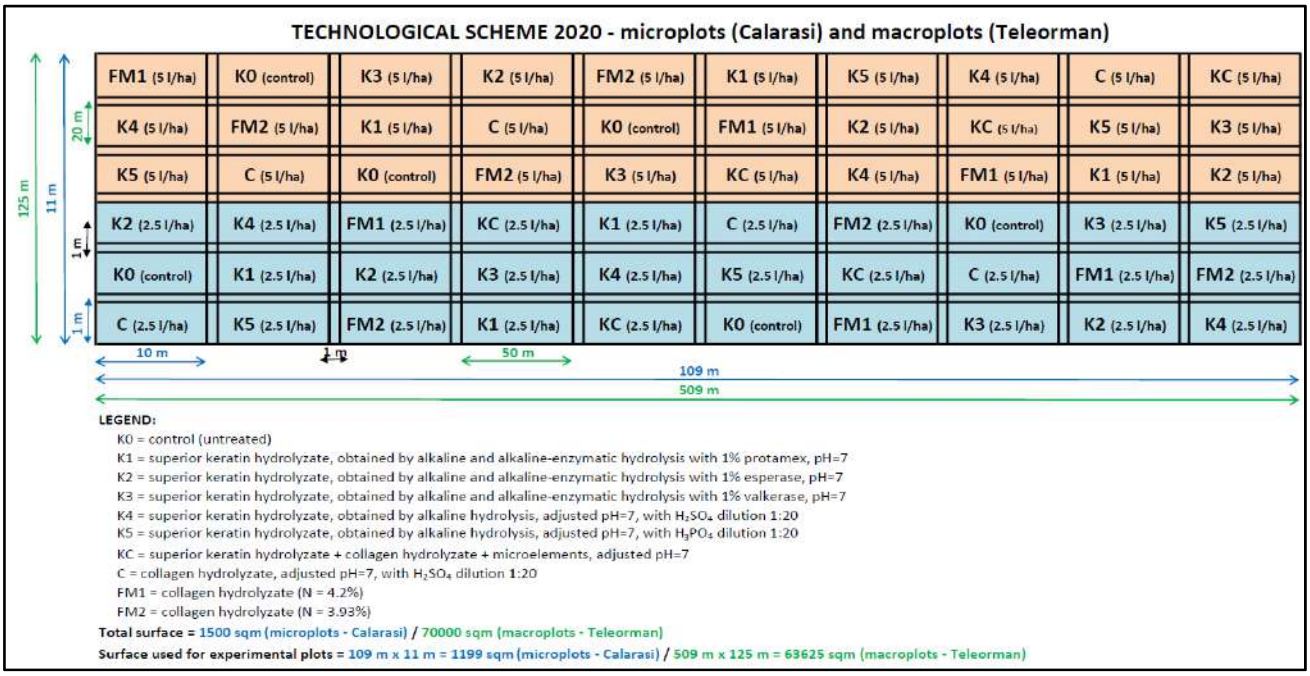


**Figure 1.** Technological scheme for the two research fields of maize

**Figure 2.** Infratec 1241 Grain Analyzer device, used for the qualitative analysis of maize grains

## Results and discussions

A short model of data management is shown in Table 3. Subsequently, all production data from Excel were entered into the Anova statistical analysis program, resulting tables and synthesis graphs.

Since in Table 3 it can be seen that the quality parameters of the yield weren't significantly influenced by the use of biostimulators based on keratin and collagen, we focused on the yield quantity from the two experimental fields.

**Table 3.** Working model in managing the results obtained after the quantitative and qualitative analysis of maize yields from 2020

| No. | Tested product | Product dose (l/ha) | U (%) | Protein (%) | Hardness (%) | Oil content (%) | WTG (g) | Yield per plot (kg) | Yield U 14% (kg) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | K0 | - | 15.3 | 9.3 | 71.2 | 3.2 | 238.85 | 5.95 | 5.86 | 5860.06 |
| 1 | K 1 | 2.5 | 16.2 | 9.6 | 70.4 | 3.0 | 248.55 | 5.82 | 5.67 | 5671.12 |
| 2 | K 1 | 5.0 | 16.7 | 9.7 | 69.5 | 3.1 | 257.05 | 5.30 | 5.13 | 5133.60 |
| 3 | K 2 | 2.5 | 16.4 | 9.7 | 70.0 | 3.0 | 233.72 | 4.80 | 4.67 | 4666.05 |
| 4 | K 2 | 5.0 | 19.4 | 9.3 | 71.8 | 3.1 | 300.56 | 5.60 | 5.25 | 5248.37 |
| 5 | K 3 | 2.5 | 19.4 | 9.6 | 70.2 | 3.2 | 221.6 | 5.87 | 5.50 | 5501.42 |
| 6 | K 3 | 5.0 | 16.2 | 9.5 | 70.8 | 3.0 | 248.08 | 5.00 | 4.87 | 4872.09 |
| 7 | K 4 | 2.5 | 15.4 | 9.5 | 71.1 | 3.4 | 409.00 | 5.99 | 5.89 | 5892.49 |
| 8 | K 4 | 5.0 | 17.4 | 10.3 | 68.1 | 3.3 | 174.15 | 7.53 | 7.23 | 7232.30 |
| 9 | K 5 | 2.5 | 15.7 | 9.3 | 71.2 | 3.3 | 259.86 | 6.90 | 6.76 | 6763.60 |
| 10 | K 5 | 5.0 | 17.5 | 9.6 | 71.0 | 3.2 | 402.7 | 6.66 | 6.39 | 6388.95 |
| 11 | KC | 2.5 | 19.9 | 9.4 | 71.7 | 3.1 | 305.8 | 5.77 | 5.37 | 5374.15 |
| 12 | KC | 5.0 | 14.9 | 10.6 | 67.5 | 3.5 | 160.35 | 6.44 | 6.37 | 6372.60 |
| 13 | C | 2.5 | 16.1 | 9.5 | 71.0 | 3.2 | 409.28 | 6.12 | 5.97 | 5970.56 |
| 14 | C | 5.0 | 15.3 | 9.9 | 69.0 | 3.0 | 171.55 | 7.31 | 7.20 | 7199.50 |
| 15 | FM 1 | 2.5 | 16.2 | 9.2 | 71.1 | 3.2 | 319.02 | 5.89 | 5.74 | 5739.33 |
| 16 | FM 1 | 5.0 | 17.4 | 9.4 | 71.1 | 3.0 | 293.1 | 5.05 | 4.85 | 4850.35 |
| 17 | FM 2 | 2.5 | 16.8 | 9.6 | 71.5 | 3.1 | 416.06 | 5.70 | 5.51 | 5514.42 |
| 18 | FM 2 | 5.0 | 17.7 | 9.6 | 70.0 | 3.4 | 235.64 | 5.26 | 5.03 | 5033.70 |

**Table 4.** Yields obtained in 2020 in microplots, on maize crop foliar fertilized with nine new biostimulators – Modelu (Calarasi)

| No. | Tested products | Dose (l/ha) | Yields (q/ha) | Control ratio (%) | Difference control (q) | Sign |
|---|---|---|---|---|---|---|
| 0 | Control | - | 54.57 | 100.00 | 0.00 | Control |
| 1. | K1 | 2.5 | 57.03 | 104.52 | 2.47 | |
| | | 5.0 | 59.80 | 109.59 | 5.23 | ** |
| 2. | K2 | 2.5 | 56.57 | 103.67 | 2.00 | |
| | | 5.0 | 58.47 | 107.15 | 3.90 | * |
| 3. | K3 | 2.5 | 54.17 | 99.27 | -0.39 | |
| | | 5.0 | 55.47 | 101.65 | 0.90 | |
| 4. | K4 | 2.5 | 61.70 | 113.07 | 7.13 | *** |
| | | 5.0 | 67.80 | 124.25 | 13.23 | *** |
| 5. | K5 | 2.5 | 56.80 | 104.09 | 2.23 | |
| | | 5.0 | 65.50 | 120.04 | 10.93 | *** |
| 6. | KC | 2.5 | 54.57 | 100.00 | 0.00 | |
| | | 5.0 | 55.27 | 101.28 | 0.70 | |
| 7. | C | 2.5 | 58.73 | 107.64 | 4.17 | * |
| | | 5.0 | 67.77 | 124.19 | 13.20 | *** |
| 8. | FM1 | 2.5 | 56.90 | 104.28 | 2.33 | |
| | | 5.0 | 58.13 | 106.54 | 3.57 | * |
| 9. | FM2 | 2.5 | 57.17 | 104.76 | 2.60 | |
| | | 5.0 | 58.20 | 106.66 | 3.63 | * |

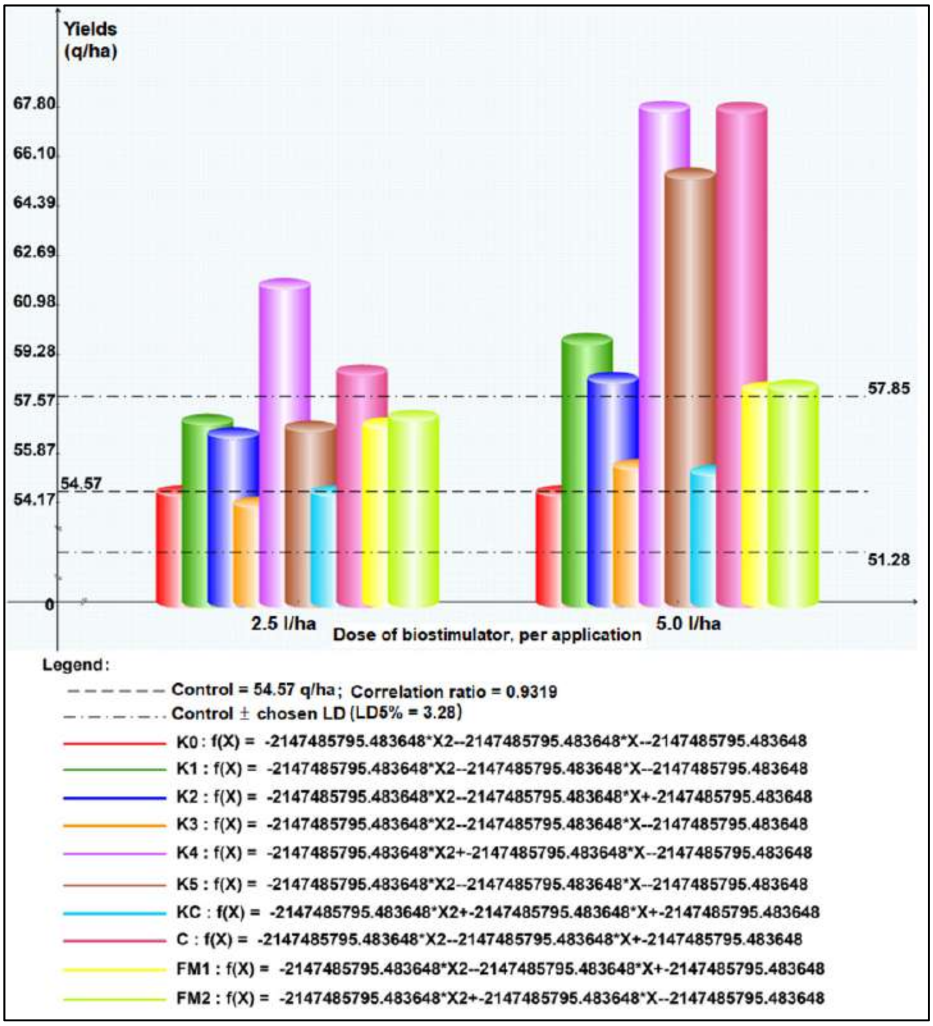


**Figure 3.** Graphic presentation of maize yields in microplots (Modelu) – bifactorial analysis between the tested products and the application doses

At Modelu (Calarasi county), in microplots of 10 sqm, due to the lack of water during the critical period of development of the maize crop (in July and August the precipitations were practically non-existent), many of the surrounding crops were compromised in proportion of 100%.

On not irrigated background, the experimental plots resisted, but with large yield losses. With a few exceptions, all the plots on which the new biostimulators have been tested obtained yields over the control versions (Table 4), to which the foliar treatments with biostimulators weren't applied, otherwise benefiting from the same agrotechnics.

Significant yield increases were found in products K4 (2.5 and 5.0 l/ha), K5 (5.0 l/ha) and C (5.0 l/ha), with values between 700 and 1300 kg/ha, and distinctly significant at K1 (5.0 l/ha), with over 500 kg/ha in addition.

Regarding the dose of 2.5 l product/ha only the biostimulators K4 (+713 kg/ha) and C (+417 kg/ha) manage to stand out, while at the double dose only K3 and KC don't exceed the confidence interval offered by the 5% difference limit.

According to this analysis, in the plots where a dose of 5.0 l product/ha has been used, there was an increase in production in each of the presented cases, with a minimum of +70 kg/ha. The graphical representation of the above results is shown in Figure 3.

Due to the better climatic conditions of this region in 2020, the experimental field located in Calomfiresti (Teleorman county), although consisting of large plots (1000 sqm) manages to obtain clearly superior yields, compared to those in microplots (Table 5).

This is also explained by the fact that in large-scale production the pedological influence is partially limited (soil unevenness, changed composition and others). Much more conclusive and compact results can be observed in terms of large-scale productions, where the positive impact of foliar treatments with biostimulators is confirmed.

Doing another exercise and excluding the applied dose factor and analyze only the 9 products under test (Table 6), compared to the untreated foliar (control), it can be seen that almost all biostimulators offer very significant yield increases (except for the KC product) – Figure 4.

**Table 5.** Yields obtained in 2020 in macroplots, on maize crop foliar fertilized with nine new biostimulators – Calomfiresti (Teleorman)

| No. | Tested products | Dose (l/ha) | Yields (q/ha) | Control ratio (%) | Difference control (q) | Sign |
|---|---|---|---|---|---|---|
| 1. | Control | - | 59.83 | 100.00 | 0.00 | Control |
| 2. | K1 | 2.5 | 68.67 | 114.76 | 8.83 | *** |
| | | 5.0 | 73.07 | 122.12 | 13.23 | *** |
| 3. | K2 | 2.5 | 68.20 | 113.98 | 8.37 | *** |
| | | 5.0 | 74.17 | 123.96 | 14.33 | *** |
| 4. | K3 | 2.5 | 60.47 | 101.06 | 0.63 | |
| | | 5.0 | 69.50 | 116.16 | 9.67 | *** |
| 5. | K4 | 2.5 | 67.23 | 112.37 | 7.40 | *** |
| | | 5.0 | 73.43 | 122.73 | 13.60 | *** |
| 6. | K5 | 2.5 | 67.33 | 112.53 | 7.50 | *** |
| | | 5.0 | 71.17 | 118.94 | 11.33 | *** |
| 7. | KC | 2.5 | 59.20 | 98.94 | -0.63 | |
| | | 5.0 | 61.50 | 102.79 | 1.67 | |
| 8. | C | 2.5 | 69.50 | 116.16 | 9.67 | *** |
| | | 5.0 | 75.20 | 125.68 | 15.37 | *** |
| 9. | FM1 | 2.5 | 67.20 | 112.31 | 7.37 | *** |
| | | 5.0 | 71.03 | 118.72 | 11.20 | *** |
| 10. | FM2 | 2.5 | 68.03 | 113.70 | 8.20 | *** |
| | | 5.0 | 69.20 | 115.65 | 9.37 | *** |

**Table 6**. Unifactorial analysis of the biostimulators' influence on maize yield

| No. | Tested products | Yields (q/ha) | Control ratio (%) | Difference control (q) | Sign |
|---|---|---|---|---|---|
| 1. | Control | 59.83 | 100.00 | 0.00 | Control |
| 2. | K1 | 70.87 | 118.44 | 11.03 | *** |
| 3. | K2 | 71.18 | 118.97 | 11.35 | *** |
| 4. | K3 | 64.98 | 108.61 | 5.15 | *** |
| 5. | K4 | 70.33 | 117.55 | 10.50 | *** |
| 6. | K5 | 69.25 | 115.74 | 9.42 | *** |
| 7. | KC | 60.35 | 100.86 | 0.52 | |
| 8. | C | 72.35 | 120.92 | 12.52 | *** |
| 9. | FM1 | 69.12 | 115.52 | 9.28 | *** |
| 10. | FM2 | 68.62 | 114.68 | 8.78 | *** |

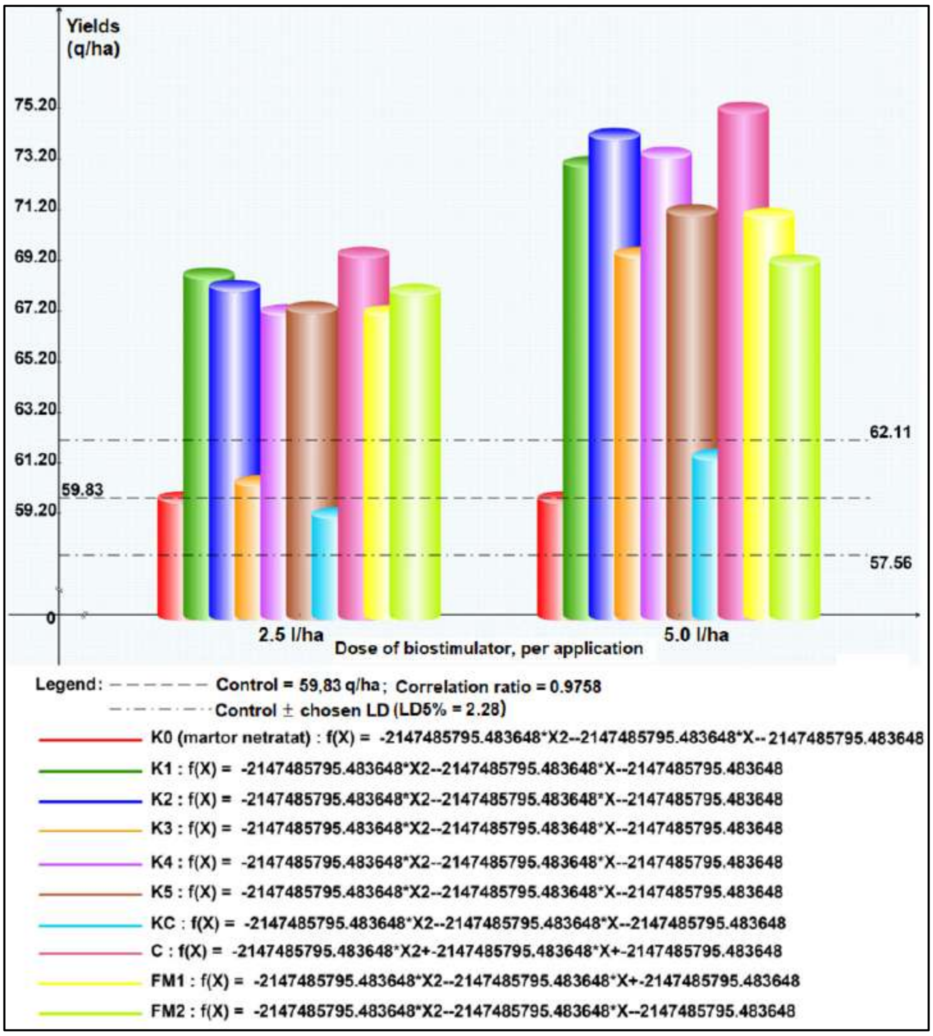


**Figure 4.** Graphic presentation of maize yields in macroplots (Calomfiresti) – bifactorial analysis between the tested products and the application doses

# Conclusion

Quantitative and qualitative results obtained in the research fields (micro- and macroplots) were contradictory, in patches, but somehow explainable, given the abnormal climatic conditions of the agricultural year 2020. However, the effects of the nine foliar biostimulators based on keratin and collagen were significant, mainly in production, in both research locations, reaching maximum yield increases of 1537 kg/ha. The only product that wasn't confirmed is KC.

Based on these results it was concluded which of the tested biostimulators has potential, and the working plan for the second year of testing was made.

## Acknowledgement

This work was supported by a grant of the Romanian Ministry of Research, Innovation and Digitalization, CCCDI – UEFISCDI, project number PN-III-P3-3.5-EUK-2017-D-0098, E!12610, FERTI-MAIZE, Contract 127/2020, within PNCDI III.

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