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BIOMETRIC RESULTS FOR MAIZE CROP, AS A RESULT OF TREATMENT WITH NEW BIOSTIMULATORS BASED ON PROTEIN ADDITIVES

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Abstract

In 2020, nine new biostimulators for maize crop, with foliar application, have been created and the testing process started. All these biostimulators are based on protein additives, respectively hydrolyzed keratin and collagen of animal origin, obtained from residues left after the skins tanning process. The tested products have been applied in 2 phases (3-4 and 7-8 leaves) and in 2 graduations (2.5 and 5.0 l/ha). In maize crop, in successive experiments in the laboratory and in research fields, two biometric indicators have been chosen, namely chlorophyll content index (CCI) and plants height. Each time the measurements have been made 5 days after the foliar application of the biostimulators. After performing both foliar treatments with biostimulators, it has been found that both chlorophyll content index (CCI) and plants height are significantly influenced by some of the products tested (increases of up to 100% in chlorophyll and 10-15% in plant height), compared to control plot (untreated). By correlating the results of these indicators with those of yields, it will be possible to choose the products with the best efficiency, with optimal characteristics for maize and which will go further in the testing process.

Key words*: maize, biostimulators, protein additives, chlorophyll, plants height.*

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important agricultural crops in the world, occupying approximately 196 million hectares worldwide (USDA, 2021). The yield potential of maize is twice as high as that of other cereals, which makes a lot of research to focus on this crop (Ali et al., 2020; Berca et al., 2014; Sible, 2019). One of the most important factors affecting crop yields is the balanced supply of essential nutrients (Fageria, 2001), i.e. those chemical elements that are absolutely necessary for plants to grow and develop.

One way to avoid problems related to availability, but also to soil pollution, is by foliar fertilization or by providing nutrients to plants through their leaves (Ali et al., 2008; Kannan, 2010; Rajasekar, 2017). The ability of plant leaves to absorb water and nutrients was recognized about three centuries ago (Alexander, 1986), and has been in the spotlight of researchers ever since.

Variable responses of field crops, including maize, to foliar applied nutrients have been and are recorded, ranging from significant yield increases to no effect (Tollenaar & Lee, 2002) and sometimes even to negative effects - decreases in yield quality and quantity (Sible, 2019). Even with variable and inconsistent results, there is a general convention that foliar application of fertilizers isn't intended to replace nutrients applied to the soil, but rather supplements soil fertility management (Halpern et al., 2015).

The newest category of products that are used to increase yields are biostimulants (Vaskova et al., 2013; Yakhin et al., 2016), with which more and more experiments are being done (Becheritu et al., 2020; Horoias et al., 2020; Pecha et al., 2011). The difference between a biostimulant and a plant growth regulator or fertilizer is vague, but biostimulants usually play a role in reducing stress or accumulating nutrients in plants (Halpern et al., 2015; Kolomaznik et al., 2012). It's assumed that these products regulate the metabolic activity of plants, in order to increase yields, which we intend to demonstrate in practice. Typically, these types of products, especially those based on collagen and keratin, both obtained from residues from slaughtered animals (Berechet et al., 2020; Niculescu et al., 2019), are aimed at relieving stress, especially drought stress.

The purpose of this paper is to analyze the reaction of maize plants to the new biostimulators, based on collagen and keratin of animal origin. According to the first year's results, the research structure for the next agricultural year will be designed.

MATERIALS AND METHODS

For the testing of the newly created products to be conclusive, it started with the laboratory stage, in which the maize plants have only been used for analyzing the first stages of vegetation, the allocated space being much too small, while pollination and fruiting practically impossible.

During the laboratory stage, the field work has been established, for which two different locations have been chosen, so that the research results to be conclusive. For both location, the same plant material has been used, namely Olt, a Romanian hybrid, group FAO 430.

In order to ensure crop's necessary nutrients, a complex fertilizer type NPK 16-16-16 (250 kg/ha), with soil incorporation, was used at the germination bed preparation. A solid fertilizer was added at the sowing time, as starter, ammonium nitrate (150 kg/ha) being used. Subsequently, only the two foliar fertilization with biostimulators were additionally performed.

The plants were foliar treated in 2 phases of vegetation, at 3-4 leaves and at 7-8 leaves.

Taking into account the particularities of the year, by observing the temperature and precipitation conditions in the first months of the year (January - April 2020), the locations for the two research fields were established (small and large plots), in different soil and climatic conditions:

→ Modelu (Calarasi county);
→ Calomfiresti (Teleorman county).

In both locations the soils are of chernozem type, richer in clay and humus in Calomfiresti (32% clay and 3.4% humus) and poorer in Modelu (27% clay and 2.9% humus). Both locations are geographically positioned in the sylvosteppe area.

All the water that falls on the ground infiltrates and forms the useful reserve of the soil, when the rains fall constantly. By applying biostimulators based on keratin and collagen on maize plants, their tolerance to drought has been followed. That is why three different environments have been chosen:

1) laboratory - potted plants, only used during the vegetation stages;
2) field (Calarasi) - microplots, of 10 sqm (1 x 10 m, 2 rows of maize each);
3) field (Teleorman) - macroplots, of 1000 sqm (20 x 50 m each).

By establishing the two fertilization graduations, the experiences became bifactorial, with the following factors (Figure 1):

1) Tested products - 9 new products + control version:

→ control;
→ K1 - superior keratin hydrolysate, obtained by alkaline and alkaline-enzymatic hydrolysis with 1% protamex, pH = 7;
→ K2 - superior keratin hydrolysate, obtained by alkaline and alkaline-enzymatic hydrolysis with 1% esperase, pH = 7;
→ K3 - superior keratin hydrolysate, obtained by alkaline and alkaline-enzymatic hydrolysis with 1% valkerase, pH = 7;
→ K4 - superior keratin hydrolysate, obtained by alkaline hydrolysis, adjusted pH = 7, with H_2SO_4 dilution 1:20;
→ K5 - superior keratin hydrolysate, obtained by alkaline hydrolysis, adjusted pH = 7, with H_3PO_4 dilution 1:20;
→ KC - superior keratin hydrolysate + collagen hydrolysate + microelements, adjusted pH = 7;
→ C - collagen hydrolysate, adjusted pH = 7, with H_2SO_4 dilution 1:20;
→ FM1 - collagen hydrolysate (N = 4.2%);
→ FM2 - collagen hydrolysate (N = 3.93%).

2) Applied doses - two graduations:

→ 2.5 l/ha;
→ 5.0 l/ha.

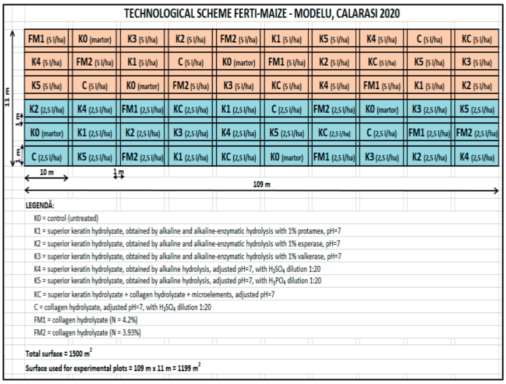

Figure 1. Technological scheme of the research field - similar for both locations

For the microplots, the products have been manually applied, with the shoulders pump, while for the macroplots the mechanized version has been used. The applications have been made in the established vegetation stages (at 3-4 and 7-8 leaves), being combined with other plant protection products (fungicide and insecticide), depending on the necessary estimated in the evaluation of the crop.

At 5 days after each of the foliar applications, 10 plants were randomly selected, for which the chlorophyll (CCI) was determined and which were measured (Figure 3). CCI (chlorophyll content index) determinations were performed using the CCM-200 Plus chlorophyll meter, produced by Opti-Sciences.

RESULTS AND DISCUSSIONS

The 2019-2020 agricultural year was a completely atypical one, with climatic conditions not at all favourable for agricultural crops. The anomalies were recorded both at the level of temperatures (above the multiannual average) and at the level of precipitation - well below the level of the multiannual average and with a deficient monthly distribution. For testing new biostimulators, however, it was the optimal environment to highlight the effects of protein additives that enter into the composition of each product based on collagen and keratin.

In February-May 2020, tests were carried out in laboratory, on potted plants, kept in the work rooms and periodically exposed to the sun (Figure 2), in similar conditions to those found in the natural environment, during the vegetation period of maize.

Figure 2. Maize plants on which biostimulators based on keratin and collage were tested, in laboratory conditions (April 2020)

Figure 3. CCI determinations in the field, on maize plants on which biostimulators based on keratin and collagen were tested (May 19, 2020)

Data were collected in large tables, then the averages were calculated and the significances were established, compared to the control variant. In Table 1 and Table 2 a data synthesis has been performed, using only the averages of the determinations collected from all the research environments. Subsequently, they were introduced in the Anova statistical program and in Excel, obtaining the graphs in Figure 4 and Figure 5, the first one for the influence of biostimulators on the level of chlorophyll in plants, and the other one for the evolution of plant height, also under the influence of the foliar fertilizations with the new products.

Table 1. Determinations of chlorophyll (CCI) in maize plants from laboratory and fields, 5 days after the application of the tested biostimulators

Tested product	Dose (l/ha)	1st application (3-4 leaves)		2nd application (7-8 leaves)	
		Laboratory	Field	Laboratory	Field
		CCI			
Control	-	11.43	12.73	11.90	18.87
K1	2.5	12.73	13.76	12.39	12.26^{o}
	5.0	12.99	14.61	20.87**	27.38**
K2	2.5	12.97	16.54	17.61*	20.54
	5.0	10.06	11.74	17.63*	21.59
K3	2.5	11.03	12.85	17.25*	22.32*
	5.0	12.54	14.39	14.93	22.11*
K4	2.5	12.15	13.50	13.39	36.03***
	5.0	11.57	13.25	10.52	23.17*
K5	2.5	11.49	14.10	10.52	22.63*
	5.0	11.13	16.39*	18.42*	22.95*
C	2.5	10.68	14.07	15.99	21.66
	5.0	13.30	13.07	19.46*	30.42***
KC	2.5	11.56	11.51	9.70	28.93**
	5.0	12.04	12.87	11.88	31.24***
FM1	2.5	13.45	13.80	10.64	18.97
	5.0	11.71	12.27	16.11	24.28*
FM2	2.5	10.89	12.79	13.79	16.02
	5.0	10.75	11.41	30.59***	31.97***
				DL5% = 6.2889	
				DL1% = 8.3757	
				DL0.1% = 10.9082	

Obviously and easy to explained are the higher values recorded in field plants, compared to those in the laboratory, which had less access to natural light.

As significance, it follows that after the first application of the new biostimulators, the differences from the control are almost imperceptible, being very close in value to it (Figure 4).

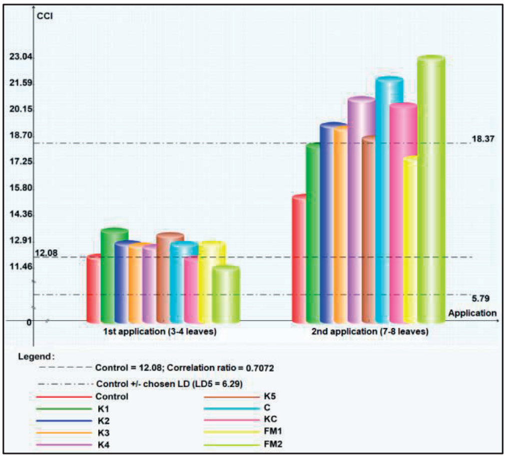

Figure 4. Chlorophyll content index (CCI) values in maize plants - laboratory and field results

The second foliar application of products with protein additives produces more visible effects, each tested product, on average for the laboratory and the field, leading to visible increases in the chlorophyll indicator. Also note the very significant positive results recorded by several products, namely K4 (2.5 l/ha), FM2 (5.0 l/ha), KC (5.0 l/ha), C (5.0 l/ha), as well as the fact that the influence of the used dose is difficult to be quantified in the case of CCI determinations. Even though CCI is an indicator that only reveals relative determinations of chlorophyll, comparing the values with the control one, the mentioned biostimulator brought a high increase, up to double.

Similarly, measurements for plant height (Table 2), another biometric parameter that was analyzed in the testing process of the new biostimulators, were made. It should be mentioned that the second measurement was performed only in the research fields, after the beginning of the reproductive stages of the maize plants, at which point the laboratory plants couldn't continue their evolution. In the table we have listed separately the values for the two experimental fields, the one with microplots (Field 1 = Calarasi) and the one with macroplots (Field 2 = Teleorman).

Table 2. Measurements of corn plant height (cm) in the laboratory and in the field

Tested product	Dose (l/ha)	1st application (3-4 leaves)		2nd application (7-8 leaves)	
		Laboratory	Field	Field 1	Field 2
		Cm			
Control	-	18.7	19.1	204.6	211.9
K1	2.5	20.6	22.8	207.8	216.0
	5.0	17.0	18.3	210.1	246.5***
K2	2.5	16.6	20.6	217.8	240.1**
	5.0	18.6	18.5	224.8	228.7*
K3	2.5	17.0	20.3	219.4	232.5*
	5.0	18.4	19.0	203.1	217.5
K4	2.5	20.5	23.6	195.6	244.4***
	5.0	14.1^{o}	19.4	192.0	241.2
K5	2.5	17.9	21.3	197.8	218.1
	5.0	14.2	20.5	219.7	242.7***
C	2.5	17.9	21.1	206.4	222.8
	5.0	19.1	23.4	226.6*	248.9***
KC	2.5	15.1	21.6	201.5	241.5
	5.0	19.6	19.0	196.0	224.8
FM1	2.5	19.5	20.5	204.8	224.5
	5.0	17.5	19.4	191.6	226.8
FM2	2.5	18.2	20.7	219.1	221.2
	5.0	14.3^{o}	17.8	235.2**	238.2**

The same trend is observed for the analysis of plant height. After the first application of the protein additives there are no visible changes, compared to the control variant. At the same time, the plants in the laboratory tend to have a lower height than those in the field, which is also easy understandable why, being the same effect as in the case of CCI parameter.

For the second application, where only measurements from the field were performed, there are very significant differences between plants, up to 50 cm, the explanation consisting in the different pedologic and climatic regime of the two research areas, as presented in the methodology.

Very significant positive compared to the control are the heights of maize plants in Teleorman (Figure 5), where the drought was more moderate, especially those fertilized with products C (5.0 l/ha), K1 (5.0 l/ha), K4 (2.5 l/ha), K5 (5.0 l/ha) and FM2 (5.0 l/ha), of which C, K4 and FM2 are common with those from chlorophyll. Also, in plant heights there are differences of about 10-15%, for almost all the fertilized plots, so this is the estimated influence for most of the tested biostimulators.

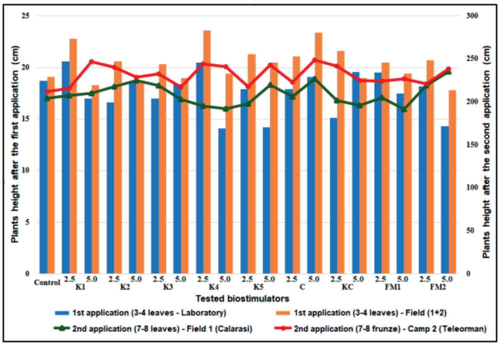

Figure 5. Height of maize plants fertilized with the new biostimulators - values from laboratory and fields

CONCLUSIONS

In 2020, the first practical researches have been carried out (in the laboratory and in the field) for the nine biostimulators under test, applied to the maize crop as foliar fertilizers.

The biometric results obtained in this first year, respectively chlorophyll (CCI) and plant height, confirmed that some protein additives have more significant effects (K1, K4, C, FM2), while others (K2, K3, FM1) don't influence the development of maize plants at all.

For confirmation, the parameters from the reproduction phases of the plants, the way in which the new products stimulate the fructification and, especially, the obtained productions were followed. The field and laboratory tests will be repeated in the next two years, for the relevance of the results.

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