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PROGRAMME RURAL DEVELOPMENT

Project "Development of innovative biostimulants for agriculture"

BG 06RDNP0001-16.001-0026, AD № RD50-62/26.02.2021

The project is funded by the EU under sub-measure 16.1 "Support for the establishment and operation of operational groups within the European Innovation Partnership" of the Rural Development Programme 2014-2020.

The activities during the second implementation period of the project cover the period from January to December 2022, including:

o the derivation of 3 precision field trials to study the effect of treating the vegetative mass of wheat, barley and hazelnut with biostimulants - samples developed by ICHT - Sofia.

Testing of three /3/ biostimulants developed by the Institute of Cryobiology and Food Technologies - Sofia in wheat, barley and hazelnut in production fields showed that these foliar biostimulants have a strong positive effect on the vegetative and generative development of the tested plants and their resistance to abiotic and biotic stress.

In the production fields of. Two field experimental trials were set up to test the effect of biostimulants with No. 1, 2, 3 and a control variant in two winter cereal crops - wheat variety Annapurna and barley variety Kalypso. The trials were laid out using the Shanin block method in 3 replications per variant, with an experimental plot size of 30 m2. A control variant was maintained for the entire growing season of the study crops, which was treated with water in an amount equal to the amount of detergent with which the other variants were treated (Scheme 1).

Scheme of the experiment

1. Control - 30 l da-1 water;

2. I variant - 30 l da-1 working solution;

3. Variant II - 30 l da-1 working solution;

4. Option III - 30 l da-1 working solution;

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The crops were sown at the optimum time, mechanically, following a sunflower precursor. With the main tillage, wheat was fertilized with 25 kg da-1 diammonium phosphate (DAP) in a single application (first disking, mid-October) and barley with 12 kg da-1 nitrogen-phosphorus fertilizer (NP) and 15 kg da-1 triple superphosphate (TSP), with two subsequent pre-sowing diskings. The sowing of cereals (wheat and barley) was carried out at the end of October.

The plot is located on black soils, the soil type reaches 100 - 110 cm. and the thickness of the humus horizon varies from 60 - 70 cm. The location of the experimental plot is in the eastern foothill region of the Balkan Mountains. Climatically, the area belongs to the temperate-continental climatic region with mild winters, wet springs, dry summers and variable autumns.

Plant treatments, in the experimental plots, were carried out at the tillering (BBCH 20-29) and flowering (BBCH 51-54) stages of wheat and barley. The treatments were applied with a backpack sprayer at a working solution rate of 30 l da-1. Immediately prior to harvest of wheat and barley, when the seeds reached the maturity stage (BBCH 92-99), 20 plants per replicate were sampled for biometric measurements. The experimental plots, per treatment, were harvested with a grain harvester and the biological yield of the crops reported. Seeds were collected from each variant for laboratory analyses.

The tested biostimulants, in wheat, have a definite influence on the structural yield elements. The highest values of these parameters were recorded in the variant with No. 2 except for plant height. The tallest plants were measured from variant No. 1 (80.10 cm) and the lowest from No. 2. The number of class-bearing stems in variant No. 2 exceeded the control by 1.8 pcs, while in the other two variants the values were close to the control. The indicator mass of class-bearing stems is similar to their number. The biostimulant applied with No. 2 also influenced the number and mass of seeds per class, contributing to their increase by 22 pc and 0.4 g, respectively, compared to the control. In spite of the reported lower values of the biometric parameters characterizing the yield, the values reported for the mass per 1000 seeds, with the three stimulants applied, exceeded those of the control (Table 1).

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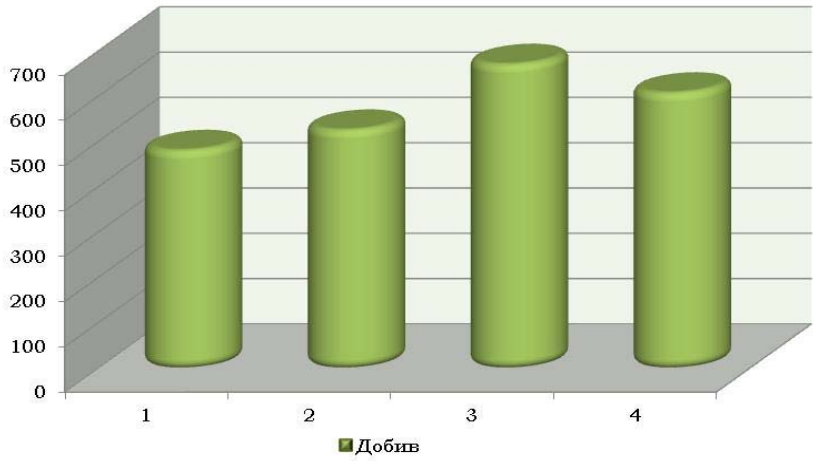
Table 1. Biometric indices in wheat variety Annapurna

Variant	Plant height cm.	Number of ear-bearing stems	Weight of ear-bearing stems, g	Number of seeds in 1 class	Weight of seeds in 1 class, g	Weight per 1000 seeds, g
1	80.10	1.35	1.79	39	1.10	43.1
2	71.40	3.70	4.12	75	1.62	45.1
3	72.25	1.80	1.91	45	1.29	44.8
4 (Control)	72.45	1.90	2.14	53	1.22	42.3

The values obtained, in wheat variety Annapurna, showed that the lowest grain yield was obtained in the control (483 kg da-1). The addition of biostimulants resulted in an increase in seed yield compared to the control, with the highest yield obtained from variant No. 2 (673 kg da-1), which exceeded the control by 39%, and in variants No. 1 and 3, by 10% (528 kg da-1)and 26% (610 kg da-1), respectively.

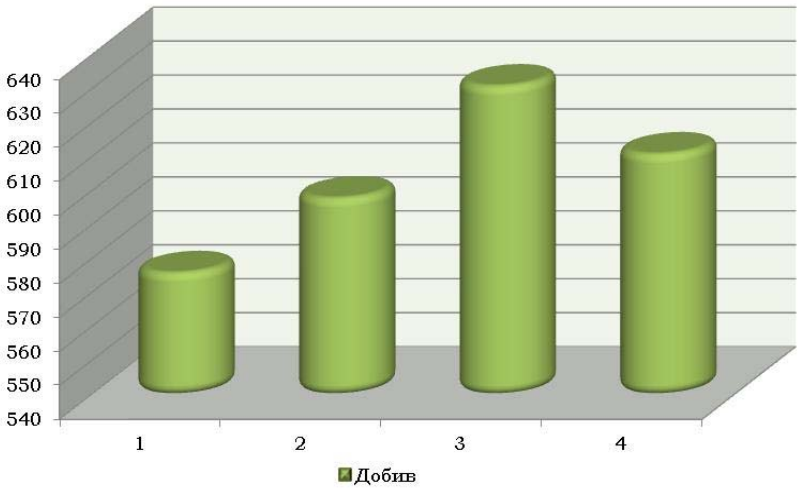
Two applications of the biostimulants during the growing season of the crops studied increased wheat yields from 10% to 39%. It also increased the mass per 1000 seeds in all the variants studied, an indicator crucial for obtaining high and stable yields.



Seed yield of wheat variety Annapurna, kg da-1

The grain yield obtained with barley variety Calypso is identical to that of wheat. The highest yield of 631 kg da-1 was obtained from the variant with biostimulant No. 2 applied, followed by No. 3 (611 kg da-1) and No. 1 (598 kg da-1). The percentage exceedance compared to the control was as follows - 9% (No. 2), 6% (No. 3) and 4% (No. 1). Biometric measurement

data corresponded with that of yield.



Seed yield of barley variety Calypso, kg da-1

Table 2. Biometric indices in barley variety Calypso

Variant	Plant height cm.	Number of ear-bearing stems	Weight of ear-bearing stems, g	Number of seeds in 1 class	Weight of seeds in 1 class, g	Weight per 1000 seeds, g
1	52.80	1.40	1.63	25	0.75	44.9
2	58.70	1.70	2.01	26	0.85	45.7
3	56.35	1.60	1.91	25	0.80	45.1
4 (Control)	50.70	1.35	1.55	24	0.73	44.2

The data shown in Figure 2 for the grain yield obtained in barley variety Calypso are identical to those of wheat. The highest yield of 631 kg da-1 was obtained from the variant with biostimulant #2 applied, followed by #3 (611 kg da-1) and #1 (598 kg da-1). The percentage exceedance compared to the control was as follows - 9% (No. 2), 6% (No. 3) and 4% (No. 1).

The biostimulator with No. 2, shows a much better effect in terms of the above indicators compared to the other two products with No. 1 and 3. This is probably due to its better balanced formulation and unique composition.

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Hazelnuts from field experience

In the spring of 2022, on an area of 4000 m2, in the hazelnut gardens of the village of Mlada gvardiya, municipality of Vetrino, Varna region, a spring hand pruning of hazelnut bushes was carried out. A field trial was built, with three organic fertilizers and a control, in four /4/ replications, with 20 shrubs per variant and 40 shrubs per control, and randomized placement of the variants. Marking of hazel bushes was carried out in April of 2022, according to a previously prepared scheme, with signs marking the exact location of the variants and their replications. The hazelnut orchard is grown organically and biologically active biostimulants were introduced between 15 and 30 May 2022 to increase productivity. The biostimulants were applied twice (leafing phase and 30 days after the first application of the stimulants), in the morning, in dry weather and at temperatures up to 22 ºC. Of the harvested products, the one with the highest yield was again the variant with biostimulant No 2 applied.

Scheme of the experiment

1. Control - 50 l da-1 water;

2. I variant - 50 l da-1 working solution;

3. Variant II - 50 l da-1 working solution;

4. Option III - 50 l da-1 working solution;

The hazelnut orchard is situated on black soils with a very good soil structure. The location of the experimental plot is in the eastern foothill region of the Balkan Mountains. Climatically, the area belongs to the temperate-continental climatic region with relatively mild winters characterised by snowfalls, wet springs and cool summers. The harvest of the marked and biostimulant-treated hazelnuts took place in October 2022.



In order to characterize the nutritional value of the produce, the total lipid content of the pooled hazelnut samples was determined to be 50.62% and the fatty acid composition.

Fatty acid composition of hazelnuts

Fatty acids	%
C14:0	0,03 %
C16:0	5,32 %
C16:1	0,30 %
C17:1	0,09 %
C18:0	3,61 %
C18:1	81,39 %
C18:2	8,74 %
C18:3	0,31 %
C20:0	0,21 %
NMC	9,17 %

NNMC	90,83 %
MNMK	81,78 %
PNMK	9,05 %
n-6/n-3	29.1 %
PNMK/NMK	0.98 %
Total lipid content:	50,62 %

In the fatty acid profile, the highest proportion is oleic acid (C18:1n-9), its content formed 81.39 % of the fatty acid composition. Although in much smaller amounts but above 1% are linoleic (C18:2n-6, 8.74%), palmitic (C16:0, 5.32%) and stearic (C18:0, 3.61%).

Hazelnuts are characterised by a low saturated fatty acid content (9.17%), with a very low percentage of myristic acid (C14:0). The latter, together with C16:0, is considered cholesterol-raising and its high content in foods is not desirable in order to reduce the risk of cardiovascular disease. Hazelnuts are characterised by a low α-linolenic acid content (C18:3n-3) of 0.31 %, which determines the n-6/n-3 fatty acid ratio of 28.19. Considering the recommendations for values of this ratio <4 (Simopoulos, 2001), the values obtained for hazelnuts are quite high, and by this indicator it could be considered that the fatty acid composition of hazelnuts is somewhat unbalanced. On the other hand, the ratio between poly- and saturated fatty acids is 0.98, which is above the recommended minimum of 0.4.

The unfavourable values of the ratio between n-6 and n-3 fatty acids is compensated by the high percentage of oleic acid. A number of scientific studies (Karacor and Cam, 2015) have highlighted the positive effect of this fatty acid on human health It is also the basis of the Mediterranean diet, whose benefits for human health have been proven.

Our results for the individual fatty acid content are in agreement with the studies of Granata et al. (2017) in hazelnuts grown in Italy and Tüfekci and Karataş (2018) in cultivars grown in Turkey, and unlike us, the latter determined linolenic acid content in the range 0.075%-0.096%. Based on these results, an assessment will be made whether foliar spraying affects the fatty acid composition balance.

In conclusion, foliar fertilization successfully corrects nutrient deficiencies during the vegetation of the plants, as it allows direct feeding. The products are formulated and fed at doses that successfully correct nutrient deficiencies and at the same time no flushing or pronounced phytotoxicity is observed.