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REACTION OF CORN HYBRIDS TO BIOSTIMULATOR APPLICATION

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Abstract. *Corn is a cereal crop with undeniable productive potential. Its great adaptability, as well as the presence of hybrids with different durations of the growing season, determine its wide distribution. Ensuring stable yields in a dynamically changing climate is proving to be a major challenge for sustainable agriculture. The current study aims to establish whether treatment with plant stimulators can help the crop to improve corn performance under drought conditions. The experiment was arranged according to the randomized complete block method in three replications and a plot size of 25 m^2. Three corn hybrids were included in the trial: DKC4949 (FAO 390), P8523 (FAO 260), and P9537 (FAO 390). The plant biostimulator Biostart was applied as a seed treatment and the product Naturamin WSP was applied twice during the vegetation period. The following parameters have been estimated: grain yield (GY); mass of grain per cob (MGC); cob weight (CW); cob length (CL); test weight (TW); 1000 grains weight (TGW) and crude protein (CP). The results showed that treatment with plant stimulants increased productivity in individual hybrids between 5% and 14%, with the highest yields realized by hybrid P8523 after Naturamine WSP application. The least pronounced is the effect of the application of Biostart in a hybrid P9537.*

Keywords*: biostimulators, corn, hybrids, productivity*

INTRODUCTION

Maize (*Zea mays* L.) is an important food, feed and industrial crop (Zhang et al., 2014). Additionally, maize is among the three most important cereal crops grown on a larger scale worldwide (Stoltz and Nadeau, 2014). Due to its high yield potential under frequent extreme weather conditions, corn is expected to provide more yields in the upcoming years (Ahmad et al., 2021). The prognoses are that half of the yield increase will be a result of changes in crop management and the other half will come from genetic improvements (Ahmad et al., 2021). Plant genetic characteristics, environment, and management practices are the most important factors influencing the crop yield (Shi et al., 2016). In maize, high population density increases the risk of logging and decreases the yield by up to 25% (Ma et al., 2014; Bian et al., 2016). Nowadays, the modern hybrids allow the farmers to improve the productivity of the crop by increasing planting density (Hernández et al., 2014; Lindsey et al., 2015) or by applying plant growth regulators (PGR) and biostimulators (Zhang et al., 2014). Plant growth stimulators are essential for the aims of sustainable agriculture and can improve crop performance in dynamically changing environmental conditions (Bailey-Serres et al., 2019). Zhang et al. (2020) observed that the lodging of corn straw occurs at or below the ear of the crop. Applying stimulators can promote the formation of biomass and high-quality corn (Ali et al., 2024). Inhibitors of plant growth are widely used by farmers to reduce the decline of grains and grain crops (Huang et al., 2017). Among the plant growth regulators, ethephon is well known due to its ability to reduce lodging (Gou et al., 2010). According to Xu et al. (2017), ethylene can reduce maize height and improve its mechanical strength. Moreover, by regulating the endogenous hormone metabolism and signaling, PGR can improve plant morphology and productivity (Peng et al., 2014; Xu et al., 2017). PGR can reduce the size of

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leaves in the upper canopy and increase the leaf angle in the middle canopy, which optimizes light distribution in the corn canopy (Naeem et al., 2012). Additionally, PGR can decrease internode length (Perez et al., 2019), which is expected to lessen competition for nutrients and enhance ear development (Cai et al., 2012; Bai et al., 2009). As a result, more assimilates can be allocated to the ear, leading to a higher grain yield (Zhao et al., 2021). The vascular bundles and cross-sectional area play a crucial role in nutrition, photosynthesis, and water movement, facilitating metabolism and transportation between roots and canopies (Huang et al., 2021) and influencing the stress resistance of stems (Rebetzke et al., 2011). Using plant growth-promoting bacteria or arbuscular mycorrhizal fungi is an effective strategy for achieving sustainable crop production (Ujvári et al., 2023). Inoculating maize with AMF typically results in increased growth (Sawers et al., 2017). Nonetheless, the extent of this effect varies based on factors such as genotype, crop rotation, tillage, and fertilization (López-Carmona et al., 2019; Sarabia et al., 2017). The present study aims to investigate the reaction of three different maize hybrids to the application of stimulators of organic origin but different compositions and to compare their effect on maize productivity.

MATERIAL AND METHODS

2.1 Field experiment

In two consecutive years, 2022 and 2023, a field experiment was set in the North-Eastern part of Bulgaria near the village of Kapinovo (43.745003° n. w. 27.991992° e. l.). The experiment included three maize hybrids: DKC4949 (FAO 390), P8523 (FAO 260), and P9537 (FAO 390). The study was arranged according to the randomized complete block design in three replications with a plot size of 25 m^2. The sowing was performed in the first 10 days of April after predecessor wheat and row spacing of 70 cm. Soil cultivation involved tilling at a depth of 8-10 cm in August after the wheat harvest, followed by plowing to a depth of 28-30 cm in October and two pre-sowing tillage sessions in the spring. Weed control was conducted using the herbicide Spectrum at a dosage of 1.3 l ha^{-1}, applied after sowing and before the crop's emergence to combat grass and some broad-leaved weeds. Following the formation of 5-6 leaves, Cambio (2 l ha^{-1}) was applied against broad-leaved weeds and Kelvin Top (1.25 l ha^{-1}) was applied against grass weeds. During the growing season, the inter-rows were cultivated twice.

The effect of two plant biostimulators have been investigated: Biostart (*Bacillus subtilis, Paenibacillus azotofixans, Bacillus megaterium, Bacillus mucilaginosus, Bacillus mycoides, Trichoderma viride* (more than 1 x 10^9 cfu/g); mycorrhiza fungie 1 x 10^7 cfu/g) applied in a dose of 0.4 g per 100 kg seeds as a seed treatment and product Naturamin WSP (free amino acids – 80%; total nitrogen – 12.8%; organic nitrogen – 12.8%) applied in a doze of 300g ha^{-1} as e leaf treatment twice in BBCH 10-19 in a distance of 10 days. The following characteristics were reported: grain yield (GY); mass of grain per cob (MGC); cob weight (CW); cob length (CL); test weight (TW); 1000 grains weight (TGW), and crude protein (CP) content.

2.2. Soil and climatic conditions

The region is represented by the chernozems soil type. The soil is loamy, and the physical clay has a share of 50%. Soil samples have been taken annually in autumn. During the study period, the soil reaction varied between 7 to 7.4. The humus content ranged between 5.3. and 6.1%. The total nitrogen content was measured according to Gurov, as the levels were

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from 53 to 57 mg per 1000 g. Phosphorus levels ranged from 0.5 to 0.9 mg per 1000 g (Egner and Rheen). Potassium content varied between 24 and 28 g per 1000 g of soil. Higher concentrations of nitrates positively impacted the physical and mechanical properties of the soil. Moreover, the humic content decreased gradually with soil depth. Despite significant amounts of nitrogen, phosphorus, and potassium, their available forms were limited. The nutritional regime of the soil improved thanks to fertilization. Before sowing, phosphorus and nitrogen fertilizers were applied at rates of 50 kg per hectare of triple superphosphate and 30 kg per hectare of ammonium nitrate.

Climatic conditions varied dynamically during the test period. The data are presented in Figure 1.

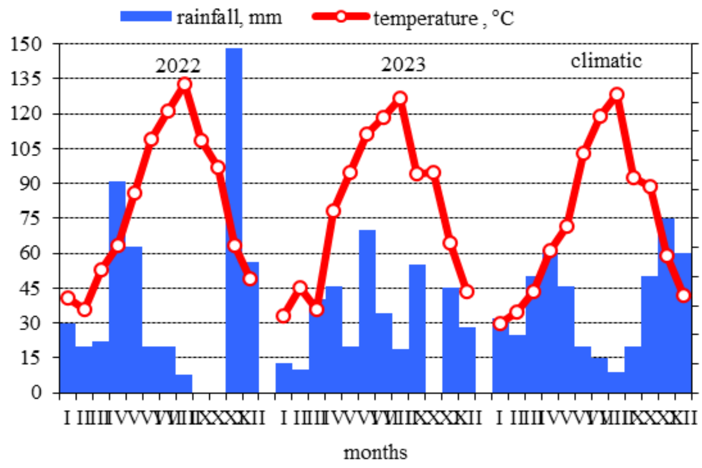

Figure 1. Climatic conditions during the study period

2.3. Statistics

The results were statistically processed using analysis of variance (ANOVA), and differences between the groups were determined through the multi-rank LSD test.

RESULTS AND DISCUSSIONS

In the first year, the yields of the untreated variants were between 10.2 t ha^{-1} for hybrid P9537 and 12.3 t ha^{-1} for hybrid P8523 (Table 1). During the same period, hybrid DKC4949 occupies an intermediate position with an average yield of 11.4 t ha^{-1}. Differences in average yields are statistically proven, and hybrids fall into separate statistical groups. The applied biostimulators increased the productivity of all hybrids. In the case of hybrid DKC4949, the action of the product Biostart contributes to achieving a maximum yield of 12.4 t ha^{-1}, but the differences between the treated variants remain unproven. By other hybrids, using Biostart contributed to an increase in average yields by 9% in hybrid P8523 and 5% in hybrid P9537. By hybrid P8523, the product Naturamin WSP increased the average yields on average with 12%, and the differences with the other variants are statistically significant and proven. While it boosts the average productivity of hybrid P9537 by about 8%, treatment with Naturamine

WSP shows no statistically significant difference compared to the control and the variant treated with Biostart.

Table 1.

Grain yield of maize

Hybrid	Treatment	Grain yield,t ha^{-1} 2022	2023	Average
DKC4949	Control	11.4[b]	11.8[a]	11.6[b]
	Biostart	12.4[c]	13.2[C]	12.8[c]
	Naturamin WSP	12.3[c]	12.9[b]	12.55[c]
P8523	Control	12.3[c]	12.6[b]	12.45[c]
	Biostart	13.4[d]	13.9[c]	13.65[d]
	Naturamin WSP	13.9[e]	14.3[d]	14.05[d]
P9537	Control	10.2[a]	10.7[a]	10.45[a]
	Biostart	10.7[a]	11.3[a]	11[a]
	Naturamin WSP	11.0[a]	11.8[a]	11.4[a]
LSD 5%		*0,83*	*1,10*	*0,97*

*Values with the same letters do not differ significantly

The second year turned out to be more favourable in climatic terms, and the average yields in the control variants increased between 3% for hybrid P8523 to 5% for hybrid P9537. In the case of the DKC4949 hybrid, the product Naturamin WSP increased the average yield by 10%, and the product Biostart by an average of 12%, and the differences between the two treatments were statistically proven. In hybrid P9537, the yields increased from 10.7 t ha^{-1} to 11.3 t ha^{-1} for the variant treated with Biostart and to 11.8 t ha^{-1} for the variant treated with Naturamine WSP. Hybrid P8523 emerged as the top performer, yielding 12.6 t ha^{-1} in the control variants. The application of biostimulators enhanced productivity by an average of 11% in the Biostart-treated variants and 14% in those treated with Naturamine WSP. Overall, during the test period, Biostart proved to be the most effective treatment by hybrid DKC4949, while Naturamine WSP delivered the best outcomes in the other two hybrids.

The independent influence of both factors, hybrid and biostimulator treatment, is presented in Table 2. The data processing showed that the hybrid is the main factor that influences the yield. During the first year the differences between the hybrids are significant, which placed them in different statistical groups. In the second year and on average for the period the productivity of hybrid P9537 is significant lower compared to the other hybrids and for this reason the hybrid is in a different statistical group. The differences between hybrid DKC4949 and P8523 remain unproven. The treatment with Naturamin WSP influnced statisticially significant increase in the grain yield for the entire period of the study.

Table 2.

Independent influence of the factors on the grain yield of maize

Factors	Source of variance	2022	2023	Average
Hybrid	DKC4949	12[b]	12.63[b]	12.32[b]
	P8523	13.2[c]	13.6[b]	12.88[b]
	P9537	10.63[a]	11.26[a]	10.95[a]
LSD	*5%*	*0.78*	*0.82*	*0.80*
	1%	*1.09*	*1.10*	*1.10*
	0.1 %	*1.15*	*1.18*	*1.17*
Treatment	Control	11.3	11.7	11.5
	Biostart	12.16[ns]	12.8[ns]	12.48[ns]
	Naturamin WSP	12.4[b]	13[b]	12.66[b]
LSD	*5%*	*0.78*	*0.82*	*0.80*
	1%	*0.94*	*1.10*	*1.10*
	0.1 %	*1.09*	*1.18*	*1.17*

[a, b, c,] significance at 5, 1, and 0,1%; [NS] non-significant

In the highly productive hybrid P8523, the yield components have the highest average values for the entire testing period, with the grain weight per cob varying between 268 and 290 g for the first and second years, respectively (Table 3 and Table 4). Treatment with plant stimulants increased the indicator by an average of up to 12%, and as a result of treatment with Naturamine WSP, the highest average grain weight per cob (330 g) was reported by hybrid P8523. For hybrid DKC4949, the values of the indicator in 2022 vary between 263 g (control) and 286 g (variant treated with Biostart), and in the second year, the values are between 275 g (control) and 308 g (variant treated with Biostart). By hybrid P9537, where the lowest average yields for the test period were reported, the values varied between 230 g (control) and 246 g (Naturamine WSP) for the first year and between 255 g (control) and 272 g (Naturamine WSP) for the second year. For the entire trial period, treatment with plant stimulants by this hybrid did not result in statistically significant differences among the various variants. The mass of the cob and the weight of 1000 seeds exhibit the same trend. By hybrid DKC4949, the highest values for both indicators were observed in the second year following treatment with Biostart. By hybrid P8523, the highest cob weight of 383 g and the highest weight per 1000 seeds of 433 g were recorded in 2023 due to treatment with Naturamin WSP. Similar results are recorded by hybrid P9537, where the application of Naturamin WSP contributed to the formation of the heaviest cobs and the largest mass per 1000 seeds. Treatment with biostimulators does not affect the test weight of individual hybrids and therefore the values varied very narrowly, as the differences between the different variants are unproven. The other indicator, which varied within narrow limits, is the length of the cob. In the first year, the values ranged from 19.8 cm by hybrid P8523 after treatment with Biostart to 23.5 cm by hybrid DKC4949 after treatment with the same product (Table 3). In the second year, there was a slight increase in the values of the indicator, as the maximum realised length of the cob of 24.8 cm was reported by hybrid P9537 after application of the product Naturamin WSP (Table 4).

Table 3.

Yield components for the first year.

Hybrid	Variant	MGC, g	CW, g	CL, cm	TW	TGW,g	CP, %
DKC4949	Control	263^{b}	330^{a}	22.6^{cb}	68.2^{a}	355^{a}	12.2^{c}
	Biostart	286^{d}	359^{c}	23.5^{c}	68.8^{a}	386^{d}	13.3^{d}
	Naturamin WSP	281^{c}	353^{c}	23.1^{c}	68^{a}	379^{c}	12.6^{cd}
P8523	Control	268^{c}	345^{b}	20.2^{a}	72.3^{c}	372^{b}	10.5^{a}
	Biostart	292^{d}	358^{c}	19.8^{a}	71.8^{c}	398^{d}	11.3^{b}
	Naturamin WSP	300^{d}	369^{de}	21.5^{b}	72^{c}	412^{e}	11.1^{ab}
P9537	Control	230^{a}	332^{a}	21.5^{b}	68.2^{a}	347^{a}	10.7^{a}
	Biostart	241^{a}	348^{b}	21.4^{b}	69.1^{a}	364^{ba}	11.3^{b}
	Naturamin WSP	246^{a}	355^{c}	22.5^{cb}	68.4^{a}	374^{b}	11^{a}
LSD 5%		*17.6*	*9.8*	*1.3*	*1.6*	*15.4*	*0.72*

*Values with the same letters do not differ significantly

For the elements of yield, the second year turns out to be more favourable, because the distribution of precipitation is more uniform and the temperature values are closer to the requirements of the crop. Regarding the crude protein content in the grains, the higher temperatures and less precipitation in the first year are also the reasons for the higher values of the indicator by all three hybrids. The applied biostimulators have a positive effect on the indicator. By the three hybrids, the product Biostart contributed to a maximum crude protein content of 11.3 % in the case of hybrids P8523 and P9537 and 13.3% by hybrid DKC4949.

Plant growth regulators can moderate crop growth depending on the production needs (Ali et al., 2024; Zhang et al., 2014). These products are widely used in the agricultural sector because their application is efficient and economically profitable (Xu et al., 2017). Research works of Ali et al. (2024) and Su et al. (2020) indicated that PGR can affect the stability and productive potential of maize by regulating the stem and ear length. According to Ali et al. (2024), RG4 treatment improved the grain yield by optimizing the leaf structure and increasing grains per row and ear. Zhang et al. (2014) reported that the grain yield was affected by PGRs application, environmental conditions, but not by the cultivar. Lower temperatures during the seedling stage in April decreased the yield by 6.7%, but the application of EDAH significantly increased maize productivity by up to 8%. The same authors observed that EDAH showed no significant effect on ear density. Moreover, the kernel number per ear was affected by the year, cultivar, plant density, as well as their interactions. Treatment with EDAH increased the

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parameter by an average of 3-4% across all years and hybrids. TKW was influenced by factors as year and plant density, while cultivar had no significant effect on it (Zhang et al., 2014). The EDAH application had the main effect on the number of kernels per ear. The application of the product increased the ear length and diameter, but the action depended on the environmental conditions. In 2017, Zhang et al. pointed out that an increased number of kernels per ear and weight as a result of DHEAP application had led to a 10.7% increase in maize yields.

Table 4.

Yield components for the second year.

Hybrid	Variant	MGC, g	CW, g	CL, cm	TW	TGW, g	CP, %
DKC4949	Control	275[b]	342[a]	23.2[ab]	70.5[a]	361[a]	11.4[c]
	Biostart	308[c]	365[c]	24.7[c]	70.8[a]	401[d]	12.2[d]
	Naturamin WSP	302[c]	359[bc]	24.5[c]	71[ab]	389[d]	11.9[c]
P8523	Control	290[b]	358[b]	22.8[a]	72.5[cb]	380[b]	9.8[a]
	Biostart	321[d]	379[e]	23.5[b]	73[c]	421[e]	10.2[a]
	Naturamin WSP	330[ed]	383[e]	22.4[a]	72.9[c]	433[f]	10[a]
P9537	Control	255[a]	346[a]	23.4[ba]	69.6[a]	355[a]	10.1[a]
	Biostart	265[a]	359[bc]	22.7[a]	70.4[a]	376[b]	10.7[b]
	Naturamin WSP	272[ab]	366[c]	24.8[c]	70.6[a]	390[c]	10[a]
LSD 5%		*18.2*	*8.7*	*0.9*	*1.4*	*13.6*	*0.78*

*Values with the same letters do not differ significantly

In the present study, it was observed that indeed the grain productivity was also influenced by the hybrid. For the tested period, hybrid P8523 was the highest yielding. The most favorable conditions in 2023 increased the productivity of the tested hybrids. The higher temperatures and less precipitation in 2022 stimulated the formation of higher protein content in the grain. According to the crude protein content, the findings of the present study are not in line with the observations of Zhang et al. (2014), where grain protein is not significantly affected by the PGR treatment. Both products contributed to the maximum values of crude protein in the grains. The bacterial product Biostart managed to achieve a higher crude protein content in all hybrids during the entire period of the study. In the study of Zhang et al. (2014), the yield increase is associated with greater kernel number per ear and greater ear size, as well as greater 1000 kernel weight, which could also be confirmed in the present work. Huang et al. (2021) analyzed the action of a novel growth stimulating product DHEAP, and discovered that its action depends mostly on the cultivar, as the effect is greater at higher plant density. The same authors reported that plant density and DHEAP treatment affected the TKW and the application of the PGR increased the parameter by 6.68%. The positive action of the biostimulators on the grain productivity elements was confirmed in the present study. Regarding the action of the biostimulators, differences are found at the hybrid level, because by two of the hybrids, Naturamin WSP has been proven to achieve the best performance, while by hybrid DKS4949, the product Biostart achieves not only maximum yield, but also contributes to the highest values of crude protein in the grains.

Bacillus strains can increase the productivity and protein content of maize (Lana et al., 2012). The product Biostart, based on *Bacillus* strains, proved its positive effect on maize productivity, as well as on the crude protein content. The stimulating effect of bacteria of the genus *Bacillus* on the growth of corn is well studied (Ahmad et al., 2008**)** together with their biocontrol activity against maize pahogenes (Szilagyi-Zecchin et al., 2014), but there is no literature data on field experiments with bacterial products and the results of their application on productivity and quality of production. The current study provides valuable data in this direction. The positive action of the product on the protein content in grains could be explained probably with increased supply of nutrients and nutrient use efficiency. Further investigation is needed to study the exact action of the product.

CONCLUSIONS

The current study found that the applied biostimulators have a positive effect on the yield and its elements by the three maize hybrids included in the study. The action of the stimulators depends on the hybrid, because hybrids coming from the same selection centre react unambiguously and achieve a better result when treated with Naturamin WSP, while hybrid DKS4949 responds better to pre-sowing treatment with the bacterial preparation Biostart. The bacterial product Biostart contributed to maximum crude protein content by all hybrids and can be recommended for sustainable agricultural production.

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