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ORIGINAL PAPER

Effect of *Bacillus* and foliar fertilization on maize yield quantity and quality*

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Abstract

The aim of the study was to evaluate the response of maize to grain inoculation with a preparation containing *Bacillus* bacteria (Primseed Biom Zboża) and / or foliar fertilization of plants (Amino Ultra Kukurydza or Plonvit Kukurydza). A field experiment was carried out in the years 2020-2022 at the Experimental Station of the Podkarpackie Agricultural Advisory Center in Boguchwala, Podkarpackie Voivodeship, Poland. A single-factor experiment was performed in four replicates in a randomized block design. The experiment was established on medium soil with a slightly acidic pH and moderate humus content. The application of *Bacillus* was shown to significantly increase plant density after emergence and before harvest. Foliar fertilizers had a positive effect on plant nutritional status (SPAD - *Soil Plant* Analysis Development) compared to the control. Application of *Bacillus* together with foliar fertilization significantly increased the number of grains per ear and TSW. However, grain yield was mainly affected by foliar fertilizers (Amino Ultra Kukurydza or Plonvit Kukurydza). As a result of their application, the grain yield exceeded 10 t ha^{-1}, while in the control, it amounted to 9.7 t ha^{-1}. The application of only Plonvit Kukurydza fertilizer had the most favorable effect on the protein content in the grain and protein yield. On the other hand, the contents of Zn and Fe in the grain were the highest after the application of Amino Ultra Kukurydza fertilizer. The application of Primseed Biom Zboża had less pronounced effect than expected.

Keywords: *Zea mays* L., microorganisms, biopreparation, nutrients, amino acids

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INTRODUCTION

Maize (*Zea mays* L.) is a cereal crop of great global economic importance, as it produces high yields and its grain or green parts can be utilized in various ways. However, this species requires optimal fertilization to meet its nutritional requirements (Jakab, Komarek 2017, Blanco-Valdes et al. 2022). Farmers usually use mineral fertilizers and pesticides to increase maize yields, which poses a threat to the natural environment. Therefore, alternative preparations are sought in modern agriculture to reduce the amount of agrochemicals used.

One such alternative is the use of microbiological preparations containing beneficial microorganisms. They have a positive effect on the growth and development of crops, especially under environmental stress conditions (Ahmad et al. 2018, Akinrinlola 2018, Coelho et al. 2021). Buzo et al. (2022) showed that the application of *Rhizoglomus intraradices* increased the uptake of nutrients, although the results were dependent on soil conditions. Wahyudi et al. (2011) and Tsotetsi et al. (2022) have argued that microbiological preparations would be increasingly used because they ensure food safety in accordance with the objectives of sustainable agricultural development. For instance, *Bacillus* spp. are already used in the production of commercial biopreparations, including those for agriculture (Pietraszek, Walczak 2014, Akinrinlola, 2018). Mumtaz et al. (2020) reported that the use of *Bacillus* in maize cultivation improved the uptake of nutrients by plants, resulting in better plant nutrition (SPAD value), increased yield, and better grain quality. Ahmad et al. (2019) confirmed that *Bacillus* application exerted a positive effect on the growth of maize plants and grain nutritional value. Similarly, Abdo et al. (2022) suggested that the negative effects of mineral fertilization could be reduced by replacing it with biological preparations or foliar fertilizers. With respect to the latter, the application of substances such as humic acids or amino acids can significantly improve maize yields. Blanco-Valdes et al. (2022) showed that seed and foliar application of Quitomax® was effective in increasing the yield and its components in maize, while González-Caballo et al. (2022) and Borak et al. (2023) have reported that foliar fertilization is already widely applied in agriculture, but it does not always ensure the expected results. This is dependent on many factors, such as the composition of the fertilizer or soil quality. Jarecki (2021) achieved the best production and economic results after multiple applications of foliar fertilization compared to a single application. Additionally, he showed that the effects of foliar fertilization were variable in the years of the study. Jakab and Komarek (2017) have demonstrated that the uptake of some micronutrients (e.g., Zn) can be hindered by excessive amounts of macronutrients (e.g., P) due to antagonism between the elements. Another study demonstrated that foliar fertilization should be adapted to each crop species, and research in this area is considered important for both scientific and agricultural practice (de Souza Júnior et al., 2022).

The purpose of the current study was to evaluate the response of maize to seed inoculation with *Bacillus* and/or foliar fertilization. The research hypothesis assumed that the optimal option in maize agriculture practice would be the combined application of *Bacillus* and foliar fertilization.

MATERIAL AND METHODS

A field experiment was carried out in the years 2020-2022 at the Experimental Station of the Podkarpackie Agricultural Advisory Center in Boguchwala, Podkarpackie Voivodeship, Poland. A single-factor experiment was performed in four replicates in a randomized block design. The tested factor was the use of a microbiological seed preparation and foliar fertilization of maize (variety Magento, FAO 240) in the following combinations:

A – Control, B – Primseed Biom Zboża, C – Amino Ultra Kukurydza, D – Plonvit Kukurydza, E – B+C, F – B+D.

The composition of the foliar fertilizer Amino Ultra Kukurydza was as follows (g kg^{-1}): boron – 16, copper – 8, iron – 68, manganese – 57, molybdenum – 0.8, zinc – 60, and the amino acid glycine. The composition of the foliar fertilizer Plonvit Kukurydza was as follows (g L^{-1}): N – 195, Mg – 15.68, SO_3 – 22, boron – 5.2, copper – 7.8, iron – 9.1, manganese – 9.1, molybdenum – 0.065, zinc – 14.3. Both foliar fertilizers were applied in three periods: BBCH 13, BBCH 18, BBCH 33. In the control, only water spraying was performed in the same amount as for foliar fertilization. Amino Ultra Kukurydza was applied at a dose of 0.5 L ha^{-1} each and Plonvit Kukurydza was applied at a dose of 2 L ha^{-1} each. The amount of working liquid for a single spray was 250 L ha^{-1}.

Primseed Biom Zboża preparation contains bacteria of the genus *Bacillus* and is intended for inoculation of seeds for sowing (0.3 L Primseed Biom Zboża + 0.9 L water per100 kg seeds). All preparations were used in doses and dates according to the manufacturer's recommendations.

The same NPK mineral fertilization was applied on the entire surface of the experimental field: 150 N kg ha^{-1}, 52.3 P kg ha^{-1} and 107.9 K kg ha^{-1}. Nitrogen dose (urea with urease inhibitor) was divided into two equal applications: pre-sowing and at the 5-leaf stage (BBCH 15). P and K fertilization was applied in the fall.

The preceding crop was winter wheat. After harvesting the preceding crop, skimming, harrowing and pre-winter plowing were performed. In the spring, harrowing was carried out and a combined cultivator was used. The area of a single plot was 15.0 m^2. Maize grains were sown to a depth of 5 cm and the width of the rows was 75 cm. Seeds were treated with the preparation Alios 300 FS (triticonazole) at a dose of 110 ml 100 kg^{-1} grain. Sowing was carried out in the third ten days of April. The seeding amount was 9 grains m^{-2} in all plots. Chemical plant protection treatments were carried out with a tractor sprayer, after prior inspection of the plantation.

The experiment was established on medium soil with a slightly acidic pH and moderate humus content. The content of assimilable phosphorus and potassium was high, and that of magnesium was medium or low (Table 1). Micronutrient content was moderate, except for low boron content. Soil sample analyses (Fotyma et al. 2015) were carried out at the District Chemical-Agricultural Station in Rzeszów (PN-ISO 10390: 1997, PB 18, 3*rd* edition of 31.07.2017, PN-R-04023:1996, PN-R-04022: 1996/Azl :2002, PN-R-04020: 1996/AzI :2004, PN-92/R-04017, PN-92/R-04016, PN-92/R-04019, PN-93/R-04021: 1994, PN-93/R-040 18).

Table 1

Chemical analysis of soil

Parameter	Unit	2020	2021	2022
pH in KCl	-	6.2	5.7	5.6
Organic carbon	(%)	0.88	0.74	0.75
P	(mg kg^{-1})	85.5	79.4	75.9
K		188.4	194.2	193.4
Mg		63	65	58
Fe		1511	2229	3079
Zn		10.5	8.8	13.0
Mn		347	430	518
Cu		4.3	5.5	6.4
B		1.2	1.0	0.9

Weather conditions were given according to the records of the meteorological station of the University of Rzeszów, located 10 km from the experimental site. The pattern of weather conditions varied over the years. High rainfall was recorded in June 2020, August 2021 and September 2022. Low precipitation occurred from May to August 2022, additionally at high air temperatures (Table 2).

Plant development stages are given according to the BBCH scale (Bundesanstalt, Bundessortenamt und CHemische Industrie). The leaf greenness index (SPAD) was measured at BBCH stage 34, using a SPAD 502P chlorophyll meter (Konica Minolta, Japan). Plant density was counted on a 1 m^2 plot after emergence and before harvest. The number of ears, grains per ear and TSW were counted on 10 randomly sampled plants from each plot. Harvesting was carried out with a field harvester at the full grain maturity stage (first ten days of October). The weight of grain from the plots was converted per hectare, with a moisture content of 14%.

The chemical composition of the grain (protein) was determined using the near-infrared method on a MPA FT-LSD Spectrometer (Bruker, Germany)

Table 2

Weather conditions

Month	Sum of precipitation (mm)				Mean temperature (°C)			
	2020	2021	2022	multi-years	2020	2021	2022	multi-years
Apr	10.0	49.4	41.8	46.0	9.2	6.5	7.3	8.7
May	83.3	63.9	2.6	77.1	11.3	12.8	15.5	13.7
Jun	162.9	47.3	0.9	80.2	18.1	18.8	20.3	17.1
Jul	18.9	55.0	11.1	95.4	18.8	21.6	20.6	19.0
Aug	7.3	107.4	10.0	65.0	19.9	17.5	21.1	18.4
Sep	43.5	85.8	116.1	62.5	15.0	13.1	12.9	13.6
Oct	54.3	2.5	32.1	46.4	11.1	9.1	10.9	8.8
Sum/Mean	380.2	411.3	214.6	472.6	14.8	14.2	15.5	14.2

in the laboratory of the Department of Plant Production at the University of Rzeszów. To determine the individual elements, the grain samples were mineralized in HNO_3: $HclO_4$: $HS2O_4$ in the ratio 20: 5: 1 in an open system in a Tecator heating block (FOSS, Denmark). The content of K, Mg, Zn, Mn, Fe and Cu in the samples was determined by atomic absorption spectroscopy (FAAS) using a Hitachi Z-2000 apparatus (Tokyo, Japan). The vanadium-molybdenum method was used to determine phosphorus on a Shimadzu UV-VIS spectrophotometer (Kyoto, Japan).

The results were statistically analyzed with the analysis of variance (ANOVA, and the Tukey's half-confidence intervals were used to determine the significance of differences between the characteristic values. Statistical analysis was performed using TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS AND DISCUSSION

The preparation containing *Bacillus* spp. significantly increased plant density after emergence and before harvest. Foliar fertilization did not modify plant density. It was shown that foliar fertilizers had a positive effect on plant nutritional status (SPAD) compared to the control. It should be noted that the SPAD measurement above 50 was obtained in variant F (Table 3).

Zakavi et al. (2022) showed in a pot experiment that *Bacillus* had a beneficial effect on maize growth under saline soil conditions. Ijaz et al. (2021) and Ahmad et al. (2022) reported that inoculation of maize kernels with *Bacillus* in combination with *Paenibacillus* significantly increased the SPAD index compared to the control, leading to enhanced plant growth. Rodrigues et al. (2021) proved that foliar fertilization improved antioxidant metabolism,

Table 3

Plant density and SPAD value

Variant	Plant density after emergence (pcs m^{-2})	Plant density before harvest (pcs m^{-2})	SPAD value
A	7.9^{b}	7.7^{b}	47.3^{b}
B	8.4^{a}	8.3^{a}	47.5^{b}
C	7.9^{b}	7.8^{b}	49.2^{a}
D	8.0^{b}	7.8^{b}	49.8^{a}
E	8.5^{a}	8.3^{a}	49.6^{a}
F	8.4^{a}	8.2^{a}	50.1^{a}
Fertilization x year	n.s.	n.s.	n.s.

A – Control, B – Primseed Biom Zboża, C – Amino Ultra Kukurydza, D – Plonvit Kukurydza, E = B+C, F = B+D. Mean values with different letters in columns are statistically different, p<0,05.

suggesting that fertilized plants were less affected by environmental stresses during their growth. Matłok et al. (2021) concluded that modern measurement techniques (e.g., NDVI) enabled rapid assessment of the nutritional status of plants and the effectiveness of applied agriculture treatments, such as fertilization.

The number of ears per plant was not significantly different between the experimental variants tested. The combined application of *Bacillus* and foliar fertilization significantly increased the number of grains per ear. When individual formulations were applied separately, the effects were less pronounced, which was confirmed statistically. The lowest number of grains per ear was developed by plants on the control plot. In addition, the combined application of *Bacillus* with one of the foliar fertilizers significantly increased TSW compared to the control and variants B and C (Table 4).

Ahmad et al. (2023) proved that inoculation of maize seeds with *Bacillus*

Table 4

Yield components

Variant	Number of cobs per plant	Number of grains per cob	Mass of 1000 grains
A	1.1^{a}	453.6^{c}	252.6^{b}
B	1^{a}	457.2^{b}	257.7^{b}
C	1.1^{a}	455.1^{bc}	258.3^{b}
D	1.1^{a}	457.7^{b}	260.4^{ab}
E	1^{a}	462.3^{a}	265.1^{a}
F	1^{a}	465.8^{a}	269.9^{a}
Fertilization x year	n.s.	n.s.	n.s.

A – Control, B – Primseed Biom Zboża, C – Amino Ultra Kukurydza, D – Plonvit Kukurydza, E = B+C, F = B+D. Mean values with different letters in columns are statistically different, p<0.05.

spp., including *Paenibacillus*, significantly increased the number of kernels per ear and TSW compared to the control. As a result, the latter authors obtained higher grain yields after the application of microbiological preparations. Blanco-Valdes et al. (2022) proved that applying biostimulants on seeds in combination with foliar fertilization significantly increased maize yield components, while Rodrigues et al. (2021) showed that foliar nitrogen fertilization increased maize yielding, as evidenced by higher TSW compared to the control. The mentioned authors believe that foliar N as stimulant fertilization in soybean crops is a feasible practice and should be seen as the new approach for obtaining plants with high metabolic activity and capable of exploiting their maximum productive potential.

Grain yield was significantly higher after application of foliar fertilizers. The yield after their application amounted to more than 10 t/ha in all variants tested. Significantly lower yields were obtained in the variant with only *Bacillus* application and in the control. Protein content in the grain was the highest after application of Plonvit Kukurydza fertilizer and significantly lower in the other variants. Protein yield was also the highest after the application of Plonvit Kukurydza fertilizer. A significantly lower result was obtained when only *Bacillus* bacteria were used and in the control (Table 5).

Ahmad et al. (2022) demonstrated that the application of *Bacillus* and *Paenibacillus* significantly increased maize yield and grain nutrient content, with average yields ranging from 8.6 to 10.1 t ha^{-1}. Blanco-Valdes et al. (2022) proved that the application of a foliar fertilizer was more effective than a seed inoculation formulation. However, they recommend the combined use of foliar and seed treatments fertilization.

Abdo et al. (2022) reported that biostimulants containing humic acids or amino acids significantly increased the size and quality of maize yields, reducing NPK fertilization by 25%. Some foliar fertilizers, in addition to macro- and micronutrients, contain other substances, such as amino acids,

Table 5

Yield and protein content in grain

Variant	Grain yield (t ha^{-1})	Protein content (% D.M.)	Protein yield (t ha^{-1})
A	9.70^{b}	9.42^{b}	0.91^{b}
B	9.78^{b}	9.43^{b}	0.92^{b}
C	10.09^{a}	9.45^{b}	0.95^{ab}
D	10.23^{a}	9.62^{a}	0.98^{a}
E	10.17^{a}	9.47^{b}	0.96^{ab}
F	10.31^{a}	9.65^{a}	0.99^{a}
Fertilization x year	n.s.	n.s.	n.s.

A – Control, B – Primseed Biom Zboża, C – Amino Ultra Kukurydza, D – Plonvit Kukurydza, E = B+C, F = B+D. Mean values with different letters in columns are statistically different, $p<0,05$.

as observed by Brankov et al. (2020) and Martínez-Gutiérrez et al. (2022); however, the efficacy of these ingredients depends on various factors, including environmental conditions. Thakur et al. (2023) showed that foliar boron fertilization had a beneficial effect on maize yield, but they obtained most beneficial results after the application of boron to the soil and three foliar applications. Khoshvaghti and Tajbakhsh-Shishavan (2022) demonstrated that foliar fertilizers had a beneficial effect on the quality of maize grain. The best results were achieved with formulations that contained not only macro- and micronutrients but also amino acids.

The preparations used in this experiment did not modify the content of macroelements in maize grain. The average contents of the analyzed elements were: P 2.05-2.13 g kg^{-1}, K 1.95-2.01 and Mg 1.15-1.24 g kg^{-1}. Zn and Fe contents were the highest after the application of Amino Ultra Kukurydza fertilizer. A significantly lower result was obtained when only *Bacillus* bacteria were used and in the control (Table 6). Similar contents of macro- and micronutrients in maize grain were obtained by Urias-Lugo et al. (2015) and Martínez-Martínez et al. (2019). In turn, Bódi et al. (2008) and Vázquez-Rodríguez et al. (2022) report higher content of elements in maize grain. Moreover, the effect of crop location was significantly greater than the effects of cultivars on concentration of Ca, P, Mg, K, Na, S, Cu, Fe, Mn and Zn.

Table 6

The chemical composition of the grain in D.M. (mean for year)

Parameter	Variant					
	A	B	C	D	E	F
P (g kg^{-1})	2.05^{a}	2.08^{a}	2.07^{a}	2.11^{a}	2.09^{a}	2.13^{a}
K (g kg^{-1})	1.95^{a}	1.99^{a}	1.96^{a}	1.98^{a}	2.01^{a}	1.99^{a}
Mg (g kg^{-1})	1.15^{a}	1.18^{a}	1.19^{a}	1.21^{a}	1.20^{a}	1.24^{a}
Cu (mg kg^{-1})	2.36a	2.40^{a}	2.43^{a}	2.41^{a}	2.44^{a}	2.42^{a}
Mn (mg kg^{-1})	5.50^{a}	5.51^{a}	5.61^{a}	5.53^{a}	5.62^{a}	5.55^{a}
Zn (mg kg^{-1})	19.22^{b}	19.28^{b}	19.41^{a}	19.31^{ab}	19.43^{a}	19.32^{ab}
Fe (mg kg^{-1})	23.30^{c}	23.40^{bc}	23.52^{a}	23.48^{ab}	23.53^{a}	23.48^{ab}

A – Control, B – Primseed Biom Zboża, C – Amino Ultra Kukurydza, D – Plonvit Kukurydza, E = B+C, F = B+D. Mean values with different letters in rows are statistically different, $p<0,05$.

CONCLUSIONS

In conclusion, the application of a preparation containing *Bacillus* bacteria yielded lower results than expected. Therefore, research on its application will be continued under environmental stress conditions. On the other hand, foliar fertilization exerted a positive effect on maize yield. Significantly lower yields were obtained in the variant with only *Bacillus* application and in the control. Plonvit Kukurydza fertilizer significantly increased grain protein content and protein yield compared to the control. On the other hand, the contents of Zn and Fe in the grain were the highest after the application of Amino Ultra Kukurydza fertilizer.

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