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Short Title: Efficiency of biofertilizers when growing corn for grain

RESEARCH ARTICLE

Efficiency of biofertilizers when growing corn for grain

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Abstract

The use of fertilizers and preparations based on bacteria while growing agricultural crops has increased significantly in recent years. Growth in demand for biological preparations is predicted due to the global economic crisis and difficulties in purchasing mineral fertilizers. Fertilizers with strains of bacteria and fungi certified by "Organic-standard" are widely available on the market. In the experiment were studied efficiency of processing corn seeds of hybrid Harmonium (FAO Euralis 380) with bio-fertilizers in a form of powder VITAMIN Q7 and as liquids LEANUM. Also, during vegetation period, corn plants were treated with a LEANUM solution of 2 l/ha (per 200 l of water). The experiment was conducted on typical low-humus medium-loam black soil on loess-like loams on the experimental field of Sumy National Agrarian University (northern forest-steppe, Ukraine). It was found that influence of biofertilizers on the elements of corn productivity depends on the weather conditions of the year. In seed treatment liquid fertilizer LEANUM is more effective than powdered analogue VITAMIN Q7. Nourishment on the variants with seed treatment and without increased the weight of 1000 grains and the number of grains per cob.

Keywords: Seed inoculation, probiotics, fertilizer, Zea mays, yield, weight of 1000 grains.

INTRODUCTION

In recent years, people have increasingly begun to research biological fertilizers or, as they are called in some literary sources, fertilizer products. As it is known, biofertilizers are environmentally friendly preparations that contain effective microorganisms and products of their vital activity (Bhattacharyya et al. 2020). Although biofertilizers with useful microorganisms were invented quite a long time ago, they began to be used more actively only after having been established by the humanity the "Goals of sustainable development" with the aim of improving ecological state of the planet. Soil probiotics "combine diverse living microbes, including bacteria, archaea, fungi, etc." (Kenawy et al. 2021).

In the process of studying biofertilizers by many scientists it was found that certain effective microorganisms affect certain biometric indices of plants, yielding capacity or soil (Datsko 2021; Sumbul et al. 2020; Nada et al. 2022).

So, for example, when studying the influence of Cupriavidus necator strain 1C2 (B1) and Pseudomonas fluorescens strain S3X (B2) on yielding capacity of corn under simulated drought conditions (the experiment was conducted under greenhouse conditions), it was found that bioinoculants increased the use of nutrients by plants, in particular nitrogen and phosphorus, and also mitigated influence of insufficient moisture (Pereira et al. 2020). By the way, the thesis that microorganisms are able to facilitate plant access to soil phosphorus is also confirmed by other sources (Rebi et al. 2022; Besharati 2018; Ahmed et al. 2020). Some studies have shown that biostimulants in combination with Pseudomonas syringae, have significant effects on corn biometrics, with significant increases in plant height, stem diameter, leaf surface area, and above- and below-ground biomass (Adoko et al. 2021).

Using probiotic Bio-Ag while growing corn, scientists have established its high effect on biological activity of the soil and stimulation of corn plants growth (Rajper et al. 2016). When using Bacillus cereus, Lactobacillus acidophilus, and Succinovibrio dextrinosolvens, their stimulating effect on corn plants growth has been proven (Santos et al. 2020).

Scientists note effectiveness of using fertilizers with bacteria and fungi when treating corn seeds, for example, with the strain

Beauveria bassiana. It is emphasized that the maximum efficiency is observed on soils/substrates with a low content of nutrients (Tall & Meyling 2018).

Recently, number of companies producing bacteria-based fertilizers has increased worldwide, and many different multicomponent mixtures have been created (García-Fraile et al. 2017; Pandey et al. 2022; Zarei 2022).

The priority is maintenance of soil fertility in proper condition, applying various science-based measures, including the use of soil pro- and prebiotics, chemical meliorants, various organic fertilizers, etc. (Boginska et al. 2019; Miransari 2017; Tamosiune et al. 2017). With application of organic farming system, increases microbiological activity of the soil, which ultimately leads to an increase in productivity elements of agricultural crops, resistance to adverse weather conditions (Rieznik et al. 2021).

Application of fertilizers certified by "Organic-standard" is safe for the environment and, of course, for humans. The use of ecologically safe products was and is important for human health, for nation health formation (Mishenin et al. 2022).

Importance of studying microfertilizers and biofertilizers in a current difficult economic and military crisis is noted by a number of scientists who emphasize the need to study different forms of fertilizers, types and terms of application. Hybrid FAO 200-299 Orzhitsa 237 MV responded well to foliar nutrition of corn plants in the phase of 3-5 leaves of BBCH 13-15, where the increase was 0.82 t/ha compared to the variant without fertilizer (Pashchak et al. 2021). In the phase of BBCH 16-18 (6-8 leaves), the increase was higher by about 30%. Even application of fertilizer in the BBCH 59 phase resulted in an increase of 1.27 t/ha, but no significant difference was found between the last two fertilizing variants.

Global warming prompts scientists to engage in the selection of corn hybrids, specific to different soil and climate zone, aimed at drought and stress resistance, etc. (Kharchenko et al. 2021; Radchenko et al. 2022; Voloshchuk et al. 2022). No less important in the technologies of crops cultivation is rational systems of fertilization and tillage, which also affects the growth and development of plants (Barczak et al. 2019; Hryhoryv et al. 2021; Tsyuk et al. 2022). The formation of the photosynthetic apparatus depends on a complex of factors, and it is possible to regulate this process by applying mineral and organic fertilizers in various ratios, forms, and doses at specified times (Lopushniak et al. 2022, Shanini et al. 2022).

Examples of positive influence of rhizobacteria affecting growth and development of plants can be listed quite a lot, so the goal of our study was to establish the influence of soil pro-prebiotics "LEANUM" and "VITAMIN O7" on yielding capacity and structure of the corn yield under conditions of Northern Forest Steppe of Ukraine.

MATERIALS AND METHODS

Characteristics of experimental plots

The experimental field is located in city Sumy on the territory of Sumy National Agrarian University, Ukraine. For growing corn for silage in 2021-2022, were used two plots located in a field with a certificate "Organic Standard". The regional climate, according to Köppen classification - Dfb, that is, a humid continental climate with warm summers and absence of difference between precipitation in different seasons. Weather conditions during vegetation period are shown in Fig. 1.

Average precipitation amount during vegetation period since 30 April 2021 till 14 September 2021 (plot 1) was equal to 252.2 mm, with the lowest average air temperature of 7.4 and the highest - 23.4 . Total precipitation amount of the second vegetation period (plot 2) since May 18, 2022 till September 9, 2022 was 218.6 mm, the lowest average temperature of this period was 11.6, and the highest - 22.3.

The predecessor is winter wheat. The soil of experimental plots is classified as a typical low-humus medium-loam black soil on the loess. The main soil tillage is flat-cut tillage to a depth of 25-28 cm. Pre-sowing tillage - cultivation to a depth of 10-12 cm. The length of experimental plots was 100 m, and the width - 12.6 m (18 rows with a row spacing 70 cm), protective plots - 2 m on each side, the total area of experimental field was 1726.4 m2. Experiments were carried out during 2021 and 2022, all necessary phytosanitary measures allowed for growing organic products were carried out in a timely manner.

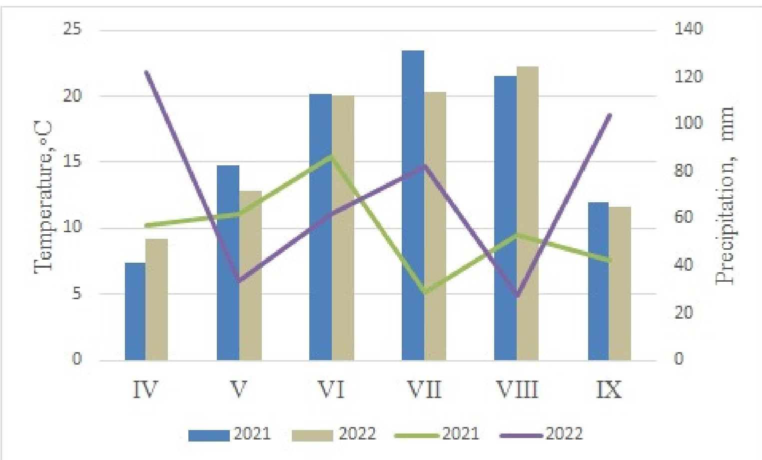

Figure 1. **Weather conditions during vegetation period since April till September**

Conducting field research in 2021-2022

In each vegetation period were carried out the same seed treatment procedures with soil pro-prebiotic "LEANUM" and "VITAMIN O7". The difference between biofertilizers is in their form: "LEANUM" is a liquid fertilizer which should be diluted with water in the proportions stated by the manufacturer, while "VITAMIN O7" is a dry fertilizer. Control variant (C) was not undergone any treatment. Before sowing corn, seed treatment was carried out 3 hours earlier in accordance with the manufacturer's recommendations. The variant with "VITAMIN O7" (V) was treated by thorough mixing in a wide container, while the variant with "LEANUM" (L) was allowed to dry before being buried in a planter in a shade. In the critical phases of the crop, treatment with "LEANUM" solution was carried out on the leaf, the first time in V5 phase, respectively, variants: C+1L, V+1L and L+1L. The second treatment was carried out with "LEANUM" solution in the V12 phase, respectively the names of the variants C+2L, V+2L and L+2L. Harmonium hybrids from Euralis with FAO 380 were used for experiments.

Gathering data and samples

Two random plants in the development phase of R8 or physiological ripeness were selected in three repetitions from each variant to determine the mass of 1000 seeds (g), the yield structure, namely length of cobs (cm), diameter of cobs (cm), number of rows (pcs) and number of grains in rows (pieces), and yielding capacity (t/ha). To determine yielding capacity the cobs from each variant were selected manually and weighed using electronic scales in field conditions. It should be added that at the same time, grain moisture was also determined using moisture meter "Fauna M". After selection of samplings in the field, measurements and calculations were carried out in the laboratory to determine yield structure, after which the cobs were peeled and the weight of 1000 seeds was weighed using scales KERN 600-2 (0.01 g).

Statistical data processing

ANOVA analysis was performed using Statistica 10.0 (StatSoft Inc., Tulsa, USA), and an LSD-test was also done.

RESULTS

Yield structure

The biggest effect on cob length during two years was recorded after seed inoculation during sowing with "LEANUM" (Tab. 1). Application of this biofertilizer in 2021 had a significant positive effect, however, this year other variants also had a significant positive effect: L+1L and L+2L, as well as the V+2L variant. In 2022, only variant L had a significant positive effect, while variants V and V+1L had significantly shorter cob length compared to the control.

Table 1. The length and diameter of corn cobs according to the variants of treatment with biofertilizers, (n=54)

	2021			2022		
Variant	Average cob length, cm x ± SD	LSD	Average cob Diameter, cm x ± SD	Average cob length, cm x ± SD	LSD	Average cob diameter, cm x ± SD
C	17.0 ± 0.4	-	4.33 ± 0.07	20.5 ± 0.7	-	4.56 ± 0.07
C+1L	18.4 ± 1.2	0.181	4.36 ± 0.07	20.5 ± 0.7	1	4.58 ± 0.04
C+2L	18.0 ± 1.0	0.351	4.35 ± 0.05	21.0 ± 0.7	0.538	4.38 ± 0.06
V	15.3 ± 0.6	0.104	4.25 ± 0.09	17.8 ± 0.5	6.2×10^{-4}*	4.36 ± 0.07
V+1L	15.2 ± 0.8	0.083	4.25 ± 0.06	18.4 ± 0.5	6.4×10^{-3}*	4.50 ± 0.03
V+2L	19.5 ± 0.3	1.9×10^{-2}*	4.23 ± 0.08	20.9 ± 0.3	0.659	4.50 ± 0.03
L	21.5 ± 0.5	9.8×10^{-5}*	4.10 ± 0.10	22.5 ± 0.3	1.0×10^{-2}*	4.61 ± 0.07
L+1L	19.8 ± 0.2	9.8×10^{-3}*	4.05 ± 0.06	19.9 ± 0.2	0.404	4.58 ± 0.08
L+2L	19.5 ± 0.5	2.3×10^{-2}*	4.21 ± 0.15	20.9 ± 0.2	0.643	4.51 ± 0.09
	$p < 0.05$		$p > 0.05$	$p < 0.05$		$p > 0.05$

Note: x - average value, SD - standard error, p - level of probability in favor of the null hypothesis; LSD - significance of the difference compared to the control according to the results of ANOVA taking into account LSD test, * - indicates a significant difference

At the same time, parameters of cob diameter were neither statistically significant in 2021 ($p = 0.22$) nor in 2022 ($p = 0.13$), so LSD-test was not performed for these parameters. On average, diameter of cobs fluctuated between 4.05 and 4.61 cm during two years.

Similarly, the number of rows in a cod had no statistically significant differences. In 2021, $p = 0.47$ and in 2022, $p = 0.11$ (Tab. 2). On average, the number of rows in a corn cob was 16, which corresponds to information from the seed originator. Instead, the number of grains in a row has a significant reliable difference. Accordingly, compared to the control, the best was treatment L before sowing in both 2021 and 2022, while treatments V and V+1L were the worst.

Table 2. Number of rows and number of grains in a corn cob according to the variants of treatment with biofertilizers, (n=54).

Variant	2021 Average number of rows in a corn cob, pcs x±SD	Average number of grains in a row of corn cobs, pcs x±SD	LSD	2022 Average number of rows in a corn cob, pcs x±SD	Average number of grains in a row of corn cobs, pcs x±SD	LSD
C	15.6±0.80	33.8±2.70	-	15.6±0.33	34.0±3.68	-
C+1L	16.3±0.61	31.8±2.07	0.527	15.3±0.42	36.1±3.65	0.43
C+2L	15.6±0.33	33.6±2.17	0.957	15.0±0.44	28.8±5.11	0.064
V	16.0±0.51	28.6±1.85	0.106	15.6±0.33	26.8±4.53	$1,1\times10^{-2}$*
V+1L	16.0±0.51	27.1±2.70	3.6×10^{-2}*	15.6±0.80	30.8±5.26	0.251
V+2L	16.3±0.33	33.3±1.11	0.874	15.3±0.42	35.5±2.94	0.584
L	15.0±0.44	39.3±2.52	0.086	14.3±0.61	38.0±5.64	0.149
L+1L	15.0±0.44	29.8±1.68	0.209	16.3±0.33	31.0±5.44	0.276
L+2L	15.6±0.33	35.8±2.62	0.527	16.3±0.33	38.3±5.39	0.118
	$p > 0.05$	$p < 0.05$		$p > 0.05$	$p < 0.05$	

Weight of 1000 seeds and yielding capacity

The greatest effect from the use of biopreparations over two years was found with C+1L, L and L+2L, these variants had a positive effect and reliable significance according to LSD-test (Tab. 3).

Table 3. Weight of 1000 corn seeds and corn yield according to the variants of treatment with bio-fertilizers

Variant	2021 Average weight of 1000 corn seeds, g x±SD	LSD	Average corn yield, t/ha x±SD	LSD	2022 Average weight of 1000 corn seeds, g x±SD	LSD	Average corn yield, t/ha x±SD	LSD
C	188,5±7,6	-	7,0±0,21	-	171,0±20,7	-	6,8±0,16	-
C+1L	243,0±8,7	$3,9\times10^{-2}$*	7,5±0,13	$3,5\times10^{-2}$*	257,4±16,8	$7,1\times10^{-3}$*	8,2±0,06	$1,0\times10^{-7}$*
C+2L	191,9±8,2	0,891	7,5±0,16	0,054	267,2±11,7	$3,3\times10^{-3}$*	9,7±0,22	$1,0\times10^{-8}$*
V	187,9±20,8	0,979	6,1±0,18	$1,5\times10^{-3}$*	179,5±34,0	0,769	6,0±0,08	$5,9\times10^{-4}$*
V+1L	191,7±18,2	0,898	6,0±0,03	$1,0\times10^{-3}$*	180,8±31,7	0,736	5,8±0,08	$1,0\times10^{-4}$*
V+2L	224,9±8,1	0,1545	7,3±0,15	0,182	225,8±17,3	0,070	7,3±0,05	$9,0\times10^{-3}$*
L	240,8±26,2	$4,6\times10^{-2}$*	8,7±0,10	$1,0\times10^{-7}$*	262,9±13,3	$4,6\times10^{-3}$*	10,0±0,19	$1,0\times10^{-8}$*
L+1L	247,4±16,5	$2,7\times10^{-2}$*	7,4±0,20	0,111	215,3±10,7	0,137	6,5±0,12	0,172
L+2L	314,5±26,6	$6,9\times10^{-5}$*	7,1±0,24	0,580	293,4±4,4	$4,2\times10^{-4}$*	7,0±0,12	0,380
	$p > 0,05$				$p > 0,05$			

Note: x - average value, SD - standard error, P - significance of the difference compared to the control according to the results of ANOVA taking into account LSD-test, * - indicates a significant difference

In 2021, a positive effect was also obtained when using L+1L, and in 2022, C+2L. Fig. 2 shows the weight of 1000 seeds, where one can see that these variants actually exceed the control by this index.

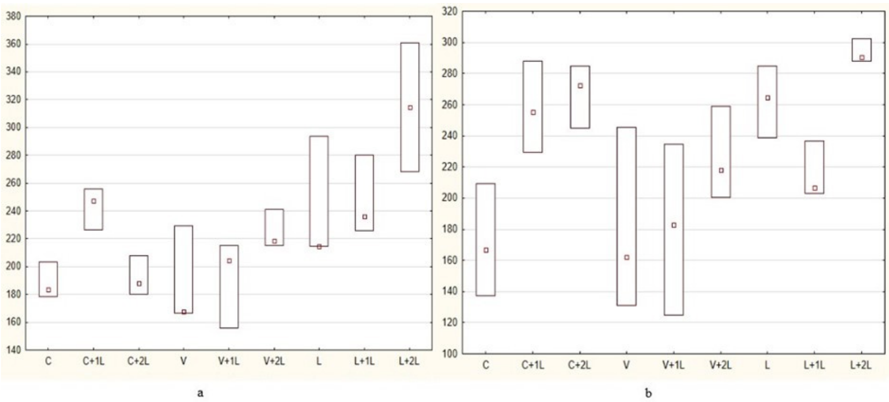

Figure2. Variability of the weight of 1000 seeds for hybrid Harmonium in relation to the variants of treatment with biofertilizers "LEANUM" and "VITAMIN O7" (n=27), where a - 2021, b - 2022: along the abscissa axis - treatment variants; along the ordinate axis - weight of 1000 seeds, g

Accordingly, corn yielding capacity is shown in Fig. 3, where one can see that the control somewhat prevails over V and V+1L by the index. Tab. 3 shows that these indices for both years are significant. This is confirmed by LSD-test. At the same time, variants C+1L and L, on the contrary, had a positive effect on corn yielding capacity in both years, while in 2022 variants C+2L and V+2L had a significant positive impact.

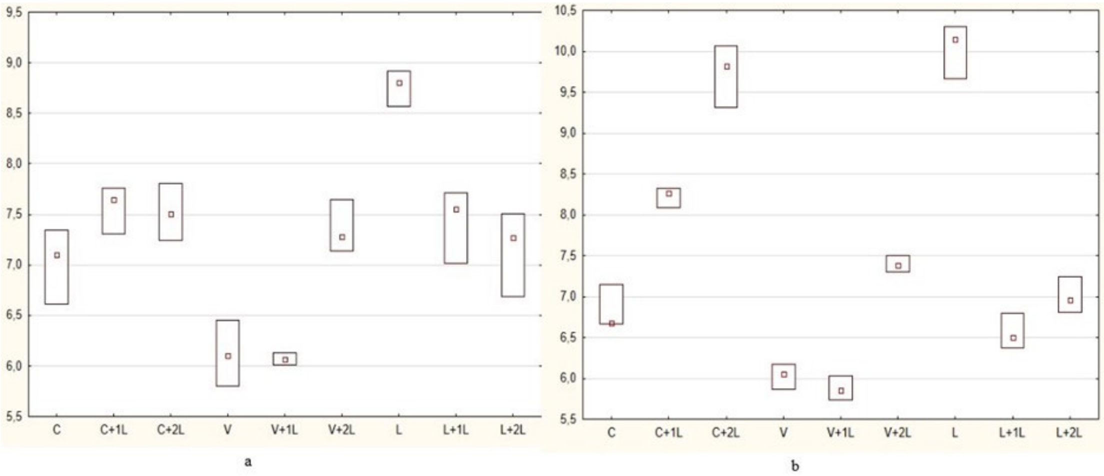

Figure. 3. Yielding capacity of hybrid Harmonium in relation to the variants of treatment with biofertilizers "LEANUM" and "VITAMIN Q7" (n=27), where a - 2021, b - 2022: along the abscissa axis – treatment variants; along the ordinate axis – yielding capacity, t/ha

DISCUSSION

In general, variant L was the best one in terms of yield structure indices. However, other variants C+1L, L, L+2L also had a positive effect on the weight of 1000 seeds and yielding capacity. Significant improvement in all analyzed indices when using L can be explained by its positive effect on the root system during seed germination, accordingly, the plants developed somewhat faster.

However, there were variants of inoculation and treatment of plants over leaves, which did not have a positive effect on the indices and also showed a significantly worse result (according to LSD-test) than the control. In general, among these variants were V and V+1L. To clarify the reasons of obtaining such results further research is needed. However, the variant V+2L showed some positive dynamics in different years according to some indices.

Similar results of positive influence of effective microorganisms during seed inoculation before sowing were obtained by (Akhtar et al. 2018), for the length of cobs, number of grains in one row, weight of 1000 seeds, and yielding capacity when these indices were studied in vegetation experiment, however, leaf treatment in this study was not conducted. Positive influence of effective microorganisms on yield structure indices was also recorded in the studies of (Nawaz et al. 2020). There was rather interesting fact - bacteria Bacillus sp. besides not having a positive effect on the cob length comparing to the control led to inhibition of its growth. Applying Enterobacter cloacae with timber waste biochar when sowing corn, (Danish et al. 2020) found significant effect on cob length and yielding capacity in dry conditions.

At the same time, (Mowafy et al., 2021) found in their research that effective microorganisms chosen by them for the study do affect the diameter of cobs, in contrast to the results obtained by our study. Research conducted in Pakistan showed that seed inoculation and foliar application of effective microorganisms is an effective means of increasing corn yielding capacity (Mubeen et al. 2021). Polish scientists also obtained evidence of increase in corn yielding capacity with seed inoculation (Mandić et al. 2018).

However, when studying effect of microbial inoculants Azospirillum brasilense ⱧMethylobacterium sp. Oliveira et al. (2022) did not find statistically significant effect on corn yielding capacity and weight of 100 seeds. Research conducted with inoculants (Salvo et al. 2018) Azospirillum brasilense or Pseudomonas fluorescens on different mineral fertilizer backgrounds led to the conclusion that “Inoculation only modified the number of microaerophilic nitrogen fixing (MNF) microorganisms at the reproductive stage of the crop”.

Effectiveness of liquid organic fertilizer "BioEcoGum" was confirmed by (Suleimenov et al. 2019). Treatment of corn seeds before sowing increased germination by 10%-30%, and application of fertilizer in foliar nutrition twice during vegetation period increased grain yielding capacity up to 75%.

Scientists note the difference in response of various hybrids and varieties of agricultural crops to probiotics used in foliar treatment. Thus, (Volpato S. et al. 2019) speaks about the lack of effect on some hybrids of corn when applying five different probiotics in foliar treatment.

CONCLUSIONS

The use of biopreparation VITAMIN Q7 in powder form for treatment of corn seeds was not effective and showed negative correlation. Seed treatment with liquid fertilizer Leanum and foliar one-time and two-time nutrition of plants contributed to the increase in corn yielding capacity. In wet vegetation period of 2022, a good result was shown by the variant without seed treatment, but with double foliar application of fertilizer. The used fertilizers did not affect cob diameter and row number in a corn cob.

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