SOILS: WHERE FOOD BEGINS

Efficiency of biological preparations based on nitrogen-fixing and phosphate-solubilizing bacteria for optimizing the plant nutrition

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Introduction

Ensuring optimal mineral nutrition of agricultural plants depends on the degree of availability of nutrients for plants. Improving the nutrition of plants only by applying of mineral fertilizers leads to undesirable environmental consequences. Soil microorganisms in the root zone are trophic mediators between the soil and the plant. Micro-organisms convert compounds inaccessible to plants into mobile forms. One of the methods for optimizing plant nutrition is pre-sowing inoculation of seeds with microbial preparations based on nitrogen-fixing and phosphate-solubilizing bacteria.

The **purpose** of research was to identify the efficacy using microbial preparations Microhumin at growing spring barley and Polymyxobacterine at growing sunflower, maize, buckwheat.

Methodology

- In the field experiment we identified the efficacy of Microhumin based on nitrogen-fixing bacteria *Azospirillum brasilense* at growing spring barley on typical chernozem in traditional farming system. The scheme was as follows: control without fertilizers; mineral fertilizers backgrounds: N_{30}; N_{60}; P_{30}; P_{60}; $N_{30}P_{30}K_{30}$; $N_{60}P_{60}$ K_{60}. And the same with the use of Microhumin. The area of the sowing plot is 100 m^2.
- Efficacy of Polymyxobacterine based on the phosphate-solubilizing bacteria *Paenibacillus polymyxa* at growing sunflower, maize and buckwheat was studied in the small-plot field experiment and at sunflower cultivation in the stationary field experiment on podzolized chernozem in organic farming system. The area of a trial plots is 1 m^2 .
- In the soil samples taken from plant root zone we identified a number of microorganisms of main ecotrophic groups by Petri dishes method and soil enzymatic activity by colorimetric method. Research plot area is 168 m^2.

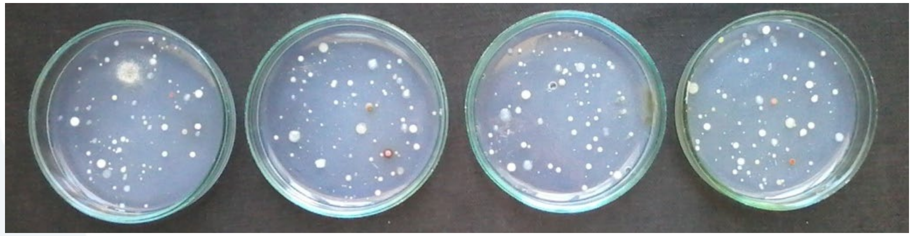
Microbiological and biochemical research methods

Number of soil microorganisms belonging to main eco-functional and taxonomic groups was determined by sowing dilutions of soil suspensions on dense nutrient media with fourfold repetition: each soil sample was sown on each nutrient medium in 4 parallel Petri dishes (ISO 7847: 2015). Total biological index (TBI) was calculated using relative values method.

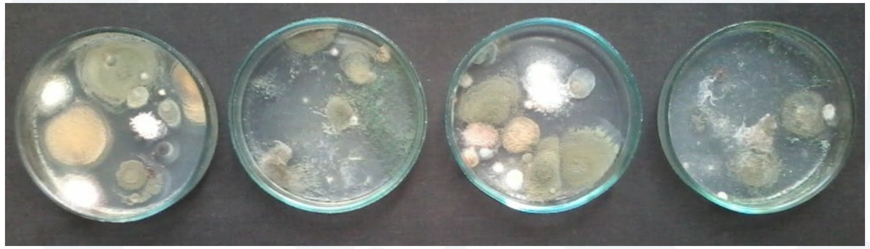
Enzymatic activity (dehydrogenase, invertase and polyphenol oxidase) was measured by photoelectrocolorimetric method.

Different ecological-trophic and taxonomic groups of microorganisms on dense nutrient media

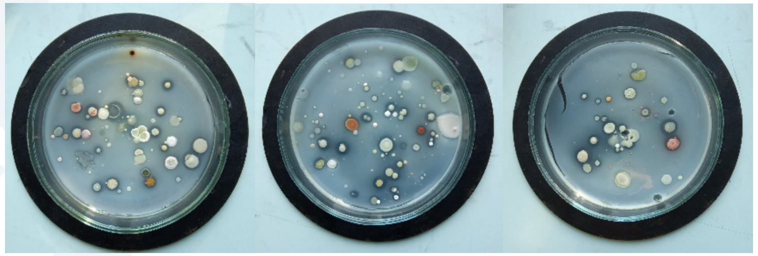
Organic nitrogen assimilating bacteria on MPA



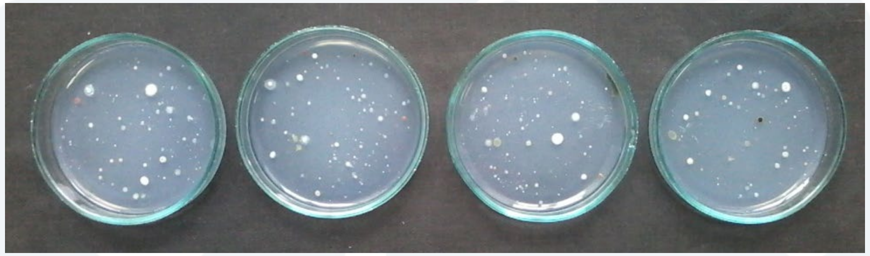
Mineral nitrogen assimilating bacteria and actinomycetes on KAA



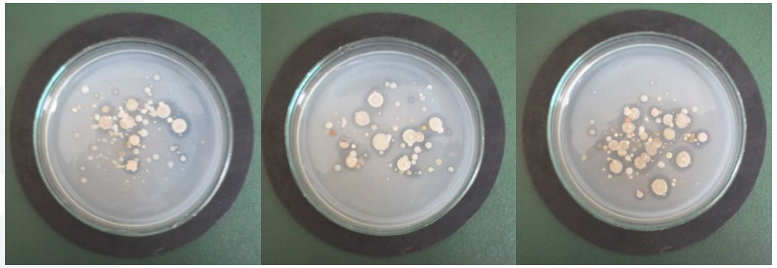
Fungi on Rihter's media



Mineral phosphate-solubilizing bacteria on Muromtsev' media



Olygotrophs on SA



Organic phosphate-solubilizing bacteria on Menkina' media

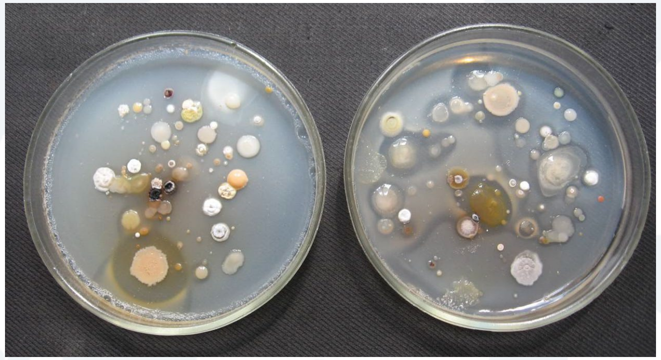
MPA - meat-peptone agar;
SAA - starch-ammonia agar;
SA – starvation agar.

Results

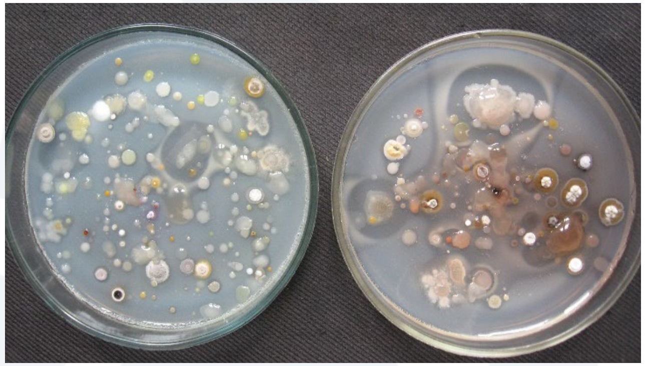
Influence of Microhumin application on the number of eutrophic and phosphate-solubilizing microorganisms in the soil of the root zone of spring barley plants on different backgrounds of mineral fertilizers

	Eutrophs, mln. CFU/g		Mineral phosphate-solubilizing bacteria, mln. CFU/g		Organic phosphate-solubilizing bacteria, mln. CFU/g	
	without inoculation	inoculation with Microhumin	without inoculation	inoculation with Microhumin	without inoculation	inoculation with Microhumin
Control	37,31	99,87	3,87	20,72	7,28	15,25
N_{30}	42,41	105,42	6,61	25,23	5,64	29,00
N_{60}	46,52	111,23	14,29	22,48	10,51	25,54
P_{60}	85,89	97,10	19,65	25,80	16,84	23,17
$N_{30}P_{30}K_{30}$	66,91	130,29	11,00	24,51	9,39	30,07
$N_{60}P_{60}K_{60}$	126,88	141,30	22,01	28,78	22,07	34,47
$LSD_{0,05}$	3,71		2,72		2,10	

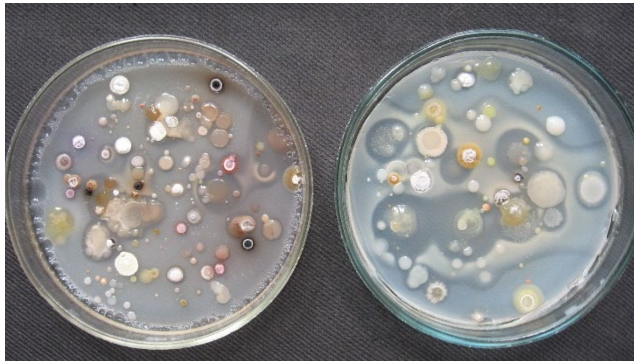
Mineral phosphate-solubilizing bacteria on the Muromtsev' medium (the middle of the growing season of spring barley)



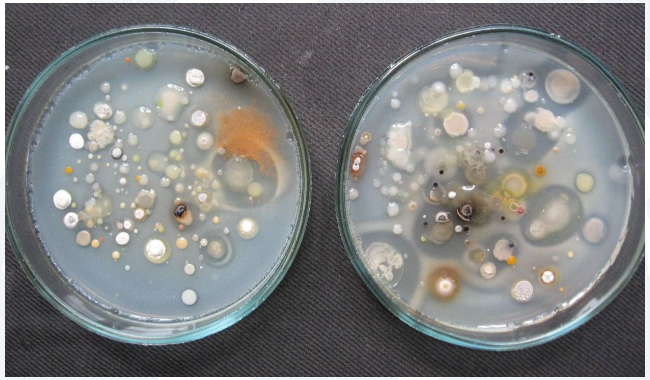
Control



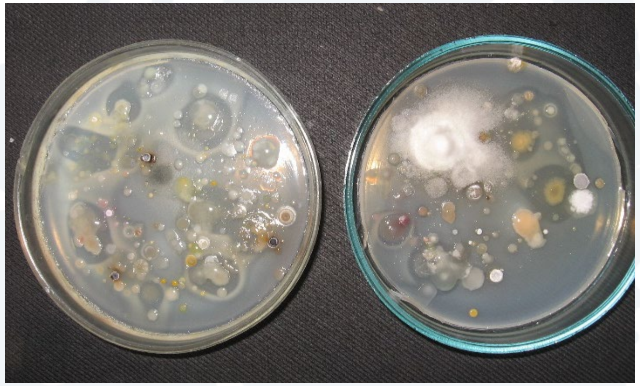
N_{30}



P_{30}



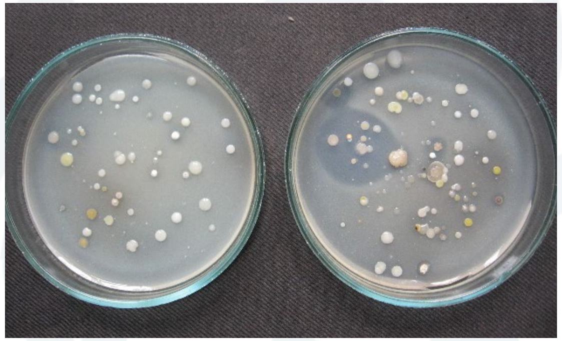
$N_{30}P_{30}K_{30}$



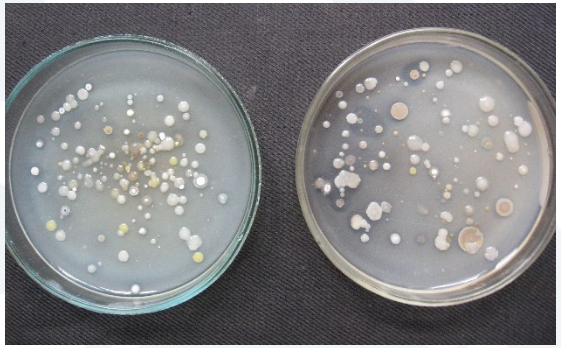
$N_{60}P_{60}K_{60}$

on the left – without inoculation,
on the right – inoculation with Microhumin

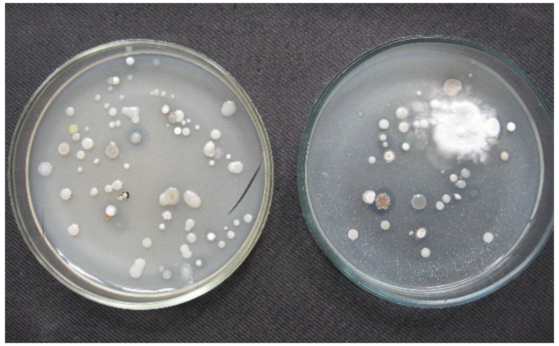
Organic phosphate-solubilizing bacteria on the Menkina’ medium (the middle of the growing season of spring barley)



Control



N_{30}



P_{30}

$N_{30}P_{30}K_{30}$

$N_{60}P_{60}K_{60}$

on the left – without inoculation,
on the right – inoculation with Microhumin

Effect of Microhumin application on spring barley yield

Variant of experiment	Without inoculation					Inoculation with Microhumin					Yield growth from the use of Microhumin on average over the study period	
	1	2	3	4	5	1	2	3	4	5	centner· ha^{-1}	%
Control	**12,7**	**3,6**	**29,5**	**14,5**	**22,5**	**16,8**	**7,1**	**41,0**	**16,7**	**25,3**	**+5,0**	**+28,6**
$N_{30}P_{30}K_{30}$	**–**	**7,0**	**35,6**	**21,2**	**26,8**	**–**	**9,4**	**47,9**	**21,6**	**29,4**	**+4,4**	**+19,5**
$N_{60}P_{60}K_{60}$	**–**	**7,1**	**41,1**	**26,2**	**27,8**	**–**	**8,6**	**51,9**	**25,8**	**30,2**	**+3,5**	**+13,7**
$LSD_{0,05}$: 1 – 0,87; 2 – 0,41; 3 – 1,57; 4 – 0,79; 5 – 1,03.												

Number of microorganisms belonging to main ecological-trophic groups in soil of plants' rhizosphere (small-plot field experiment; at the beginning of the growing season)

Crops	Variant of experiment	Nitrogen assimilating microorganisms, mln. CFU/g				Fungi, th. CFU/g	Olygo-trophs, mln. CFU/g	Eutrophs, mln. CFU/g	Indices			
		organic	mineral						oligotro-phicity	minera-lization	MTSOM	TBI, %
			total	bacteria	actino-mycetes							
sunflower	Control	16.25	24.26	14.42	9.84	70.56	24.26	40.58	0.60	1.49	27.19	63
	Polymyxobacterine	24.93	43.98	26.58	17.40	52.78	32.22	68.96	0.47	1.76	39.15	100
buckwheat	Control	17.09	32.96	22.56	10.40	60.44	30.72	50.11	0.61	1.93	25.93	79
	Polymyxobacterine	18.80	35.02	22.03	12.99	73.67	36.52	53.89	0.68	1.86	28.94	94
maize	Control	19.80	35.12	27.87	7.25	83.04	24.27	55.00	0.44	1.77	31.03	69
	Polymyxobacterine	32.27	47.34	39.03	8.31	60.23	33.28	79.68	0.42	1.47	54.16	100
$LSD_{0,05}$		4.19	4.83	–	2.24	21.97	470	–	–	–	–	–

CFU – colony forming units;
MTSOM – coefficient of microbial transformation of soil organic matter;
TBI – total biological index;
LSD – least significant difference.

Number of microorganisms belonging to main ecological-trophic groups in soil of plants' rhizosphere (small-plot field experiment; at the end of the growing season)

Crops	Variant of experiment	Nitrogen assimilating microorganisms, mln. CFU/g				Fungi, th. CFU/g	Olygo-trophs, mln. CFU/g	Eutrophs, mln. CFU/g	Indices			
		organic	mineral						oligotro-phicity	minera-lization	MTSOM	TBI, %
			total	bacteria	actino-mycetes							
sunflower	Control	16.32	39.24	25.14	14.10	65.18	91.20	55.63	1.64	2.40	23.15	73
	Polymyxobacterine	17.61	42.26	28.52	13.74	64.06	68.97	59.93	1.15	2.39	24.94	82
buckwheat	Control	32.00	59.26	45.10	14.16	74.15	102.80	91.33	1.13	1.85	49.33	81
	Polymyxobacterine	25.84	63.72	51.33	12.39	61.66	71.98	89.62	0.80	2.47	36.32	82
maize	Control	14.51	43.66	32.10	11.56	65.77	49.74	58.24	0.85	3.01	19.33	57
	Polymyxobacterine	24.34	52.65	44.58	8.07	61.39	109.80	77.05	1.43	2.16	35.64	96
$LSD_{0,05}$		4.78	6.49	–	2.38	10.59	11.44	–	–	–	–	–

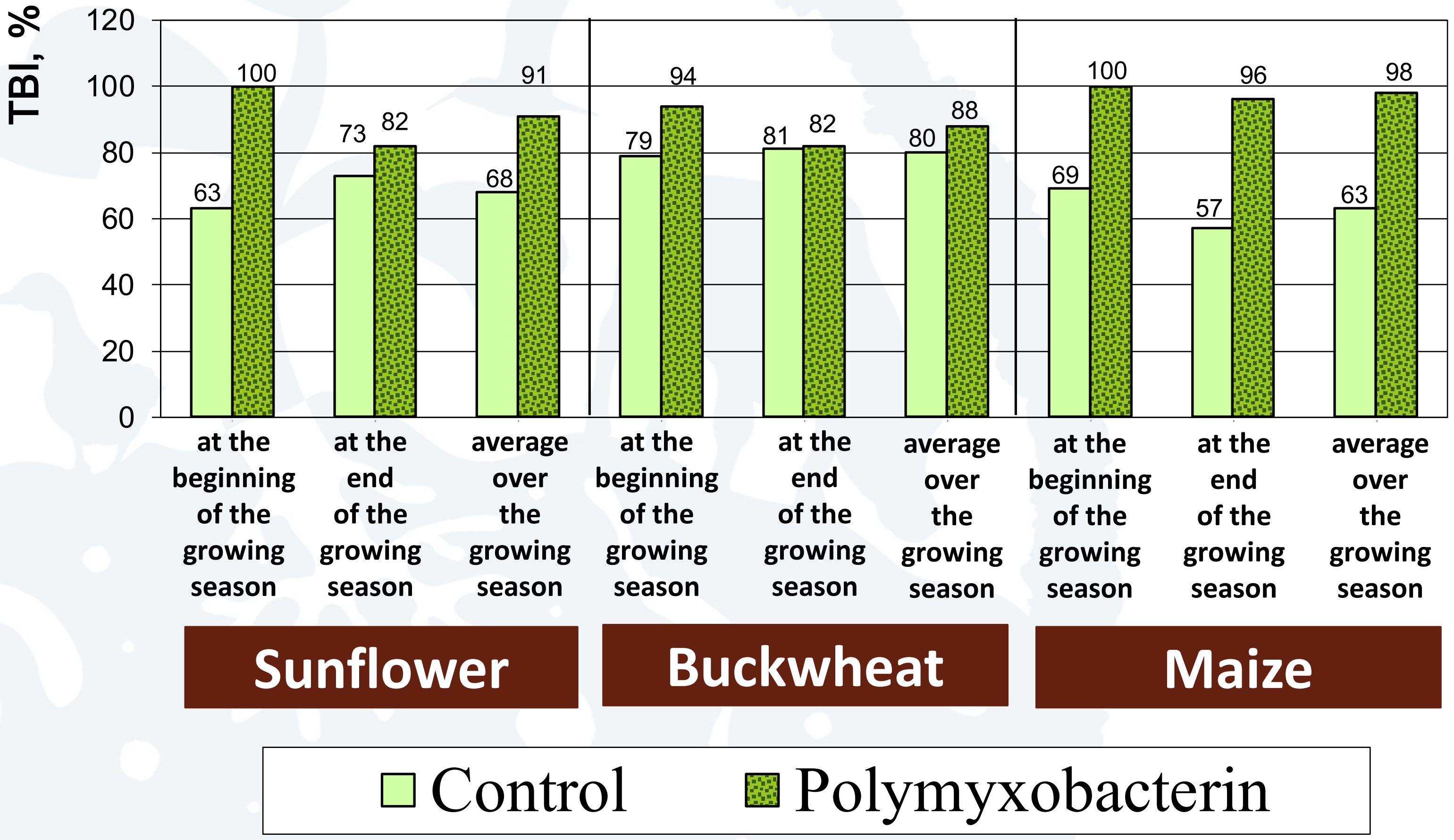
CFU – colony forming units;
MTSOM – coefficient of microbial transformation of soil organic matter;
TBI – total biological index;
LSD – least significant difference.

Number of microorganisms belonging to main ecological-trophic groups in soil of plants' rhizosphere (small-plot field experiment; numbers are average over the growing season)

Crops	Variant of experiment	Nitrogen assimilating microorganisms, mln. CFU/g				Fungi, th. CFU/g	Olygo-trophs, mln. CFU/g	Eutrophs, mln. CFU/g	Indices			
		organic	mineral						oligotro-phicity	minera-lization	MTSOM	TBI, %
			total	bacteria	actino-mycetes							
sunflower	Control	16.29	31.75	19.78	11.97	67.87	57.73	48.11	1.20	1.95	24.64	68
	Polymyxobacterine	21.27	43.12	27.55	15.57	58.42	50.60	64.45	0.79	2.02	31.88	91
buckwheat	Control	24.55	46.11	33.83	12.28	67.30	66.76	70.72	0.94	1.88	37.59	80
	Polymyxobacterine	22.32	49.37	36.68	12.69	67.67	54.25	71.76	0.76	2.21	32.44	88
maize	Control	17.16	39.39	29.99	9.41	74.41	37.01	56.62	0.65	2.30	24.59	63
	Polymyxobacterine	28.31	50.00	41.81	8.19	60.81	71.54	78.36	0.91	1.77	44.24	98
$LSD_{0,05}$		4.19	4.63	–	2.24	10.59	4.70	–	–	–	–	–

CFU – colony forming units;
MTSOM – coefficient of microbial transformation of soil organic matter;
TBI – total biological index;
LSD – least significant difference.

Influence of Polymyxobacterine application on the state of microbial cenosis in soil of plants' root zone



Effect of Polymyxobacterine on soil enzymatic activity in the plants' root zone

Crops	Variant of experiment	Dehydrogenase, mg TPF / 100 g per 24 hours			Invertase, mg glucose / g per 24 hours			Phenol oxidase, mg 1,4-n-benzoquinone / 10 g per 1 hours		
		at the beginning of the growing season	at the end of the growing season	average over the growing season	at the beginning of the growing season	at the end of the growing season	average over the growing season	at the beginning of the growing season	at the end of the growing season	average over the growing season
sunflower	Control	200,00	178,00	189,00	5,77	11,11	8,44	752,00	1296,00	1024,00
	Polymyxobacterine	254,40	171,70	213,05	10,42	8,84	9,63	842,70	922,20	888,45
buckwheat	Control	147,10	217,80	182,45	8,20	7,52	7,86	1020,40	1064,70	1042,55
	Polymyxobacterine	207,20	209,20	208,20	6,84	7,39	7,12	633,60	936,20	784,90
maize	Control	138,00	217,80	177,90	8,69	8,31	8,50	1131,20	1081,20	1106,20
	Polymyxobacterine	123,20	210,60	166,90	8,34	6,29	7,32	938,40	906,30	922,35
$LSD_{0,05}$		20,83	24,03	–	0,08	0,26	–	30,33	41,60	–

Influence of seed presowing treatment by the biological preparation on a growth and development of sunflower plants

Control

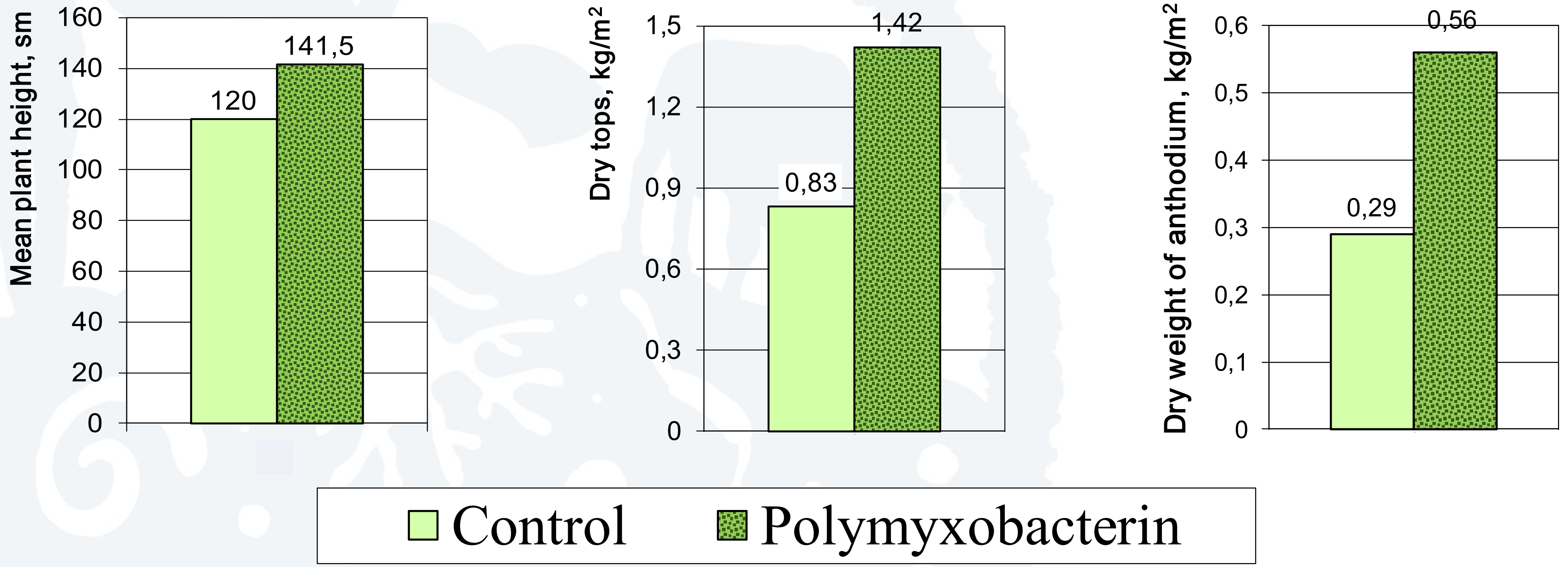
Polymyxobacterine

left – control

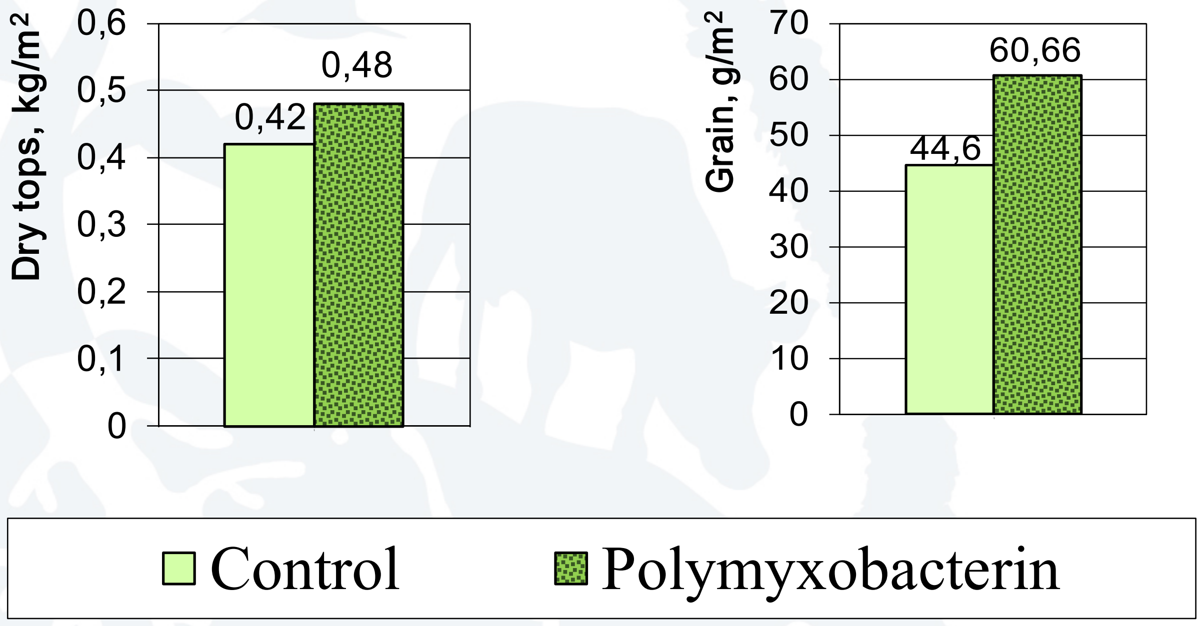
right – with Polymyxobacterin

The root system of corn plants

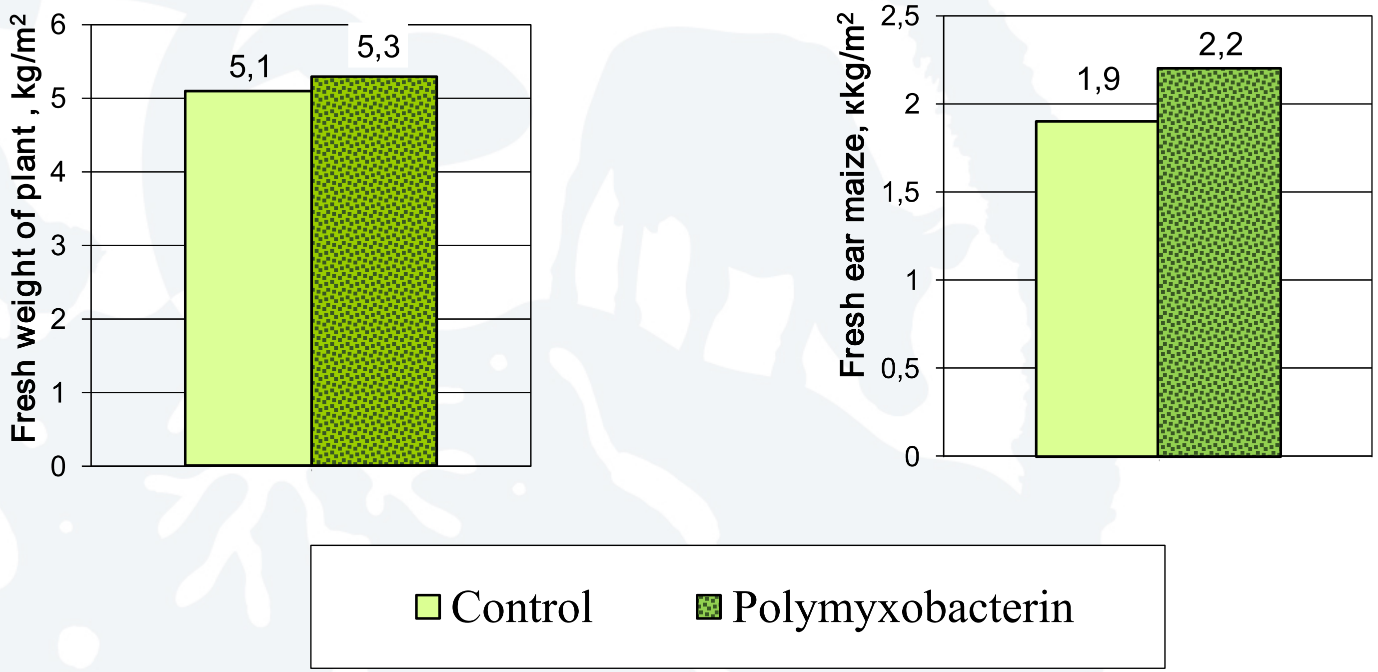
Biometric data and crop of sunflower



Crop of buckwheat



Crop of maize



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Quality of maize grain

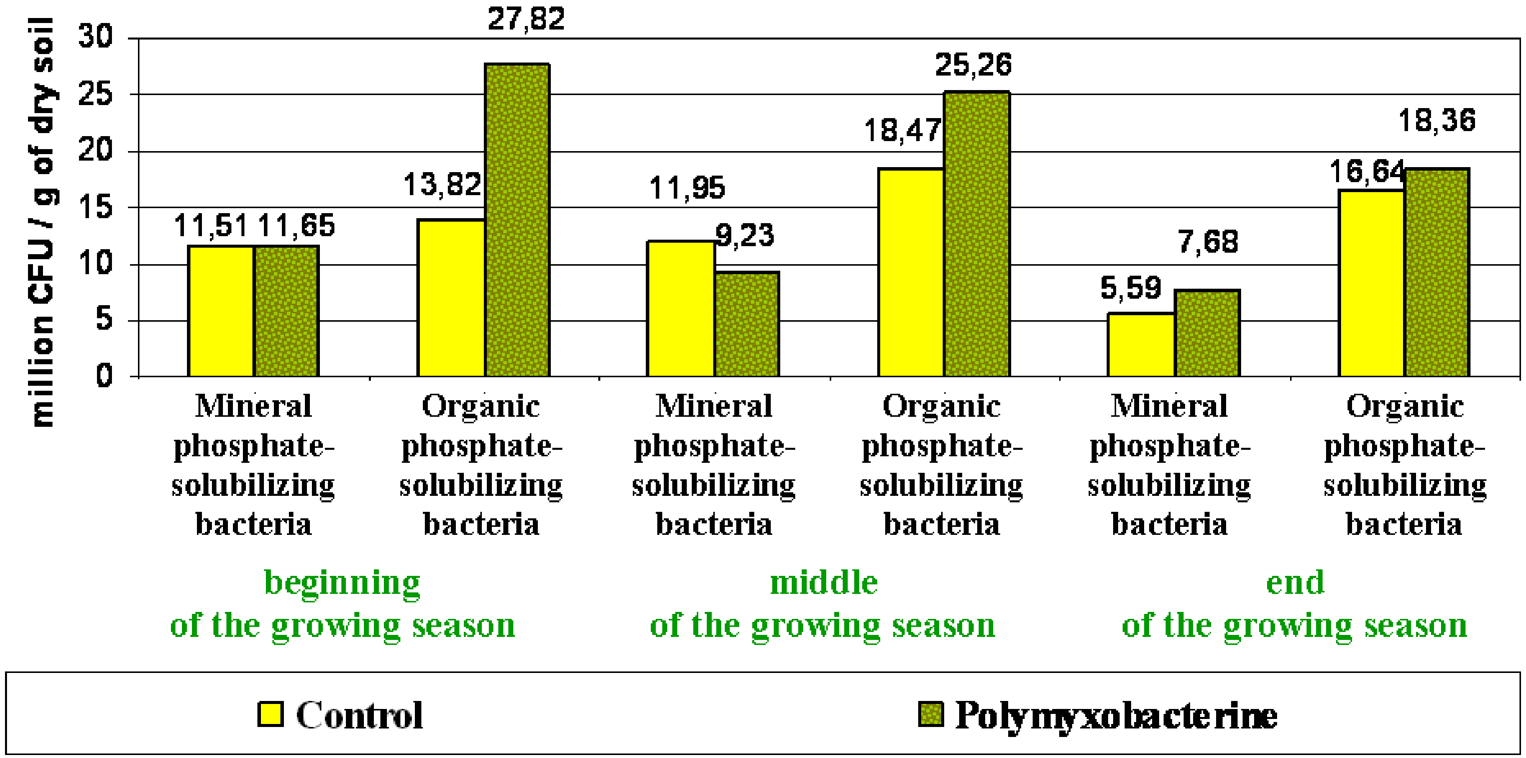
Variant of experiment	Protein, %	Oil, %	Cellulose, %	Leach, %	N, %	P_2O_5, %	K_2O, %
Control	**6.42**	**2.39**	**1.70**	**1.01**	**1.44**	**0.75**	**0.33**
Polymyxobacterine	**7.06**	**3.28**	**2.16**	**1.07**	**1.56**	**0.78**	**0.36**
Least significant difference $_{0,05}$	0.74	0.56	0.50	0.05	0.02	–	–

Impact of Polymyxobacterine on the number of microorganisms in the soil of sunflower plants’ root zone under conditions of organic farming system (long-term stationary field experiment)

Variant of experiment	Humidity of soil, %	Nitrogen assimilating microorganisms, mln. CFU/g				Fungi, th. CFU/g	Olygotrophs, mln. CFU/g	Eutrophs, mln. CFU/g	Indices			
		organic	mineral						oligotrophicity	mineralization	MTSOM	TBI, %
			total	bacteria	actinomyces							
At the beginning of the growing season												
Control	18.45	13.17	38.10	27.07	11.03	49.47	17.13	51.32	0.33	2.89	17.72	100
Polymyxobacterine	17.90	17.90	47.32	30.72	16.60	63.08	17.97	65.28	0.28	2.64	24.67	126
LSD $_{0,05}$	–	1.41	4.37	–	0.62	8.31	3.95	–	–	–	–	–
At the end of the growing season												
Control	18.33	13.28	47.14	39.71	7.43	28.69	24.66	60.45	0.41	3.55	17.02	100
Polymyxobacterine	19.88	16.20	51.24	43.08	8.16	23.08	33.12	67.46	0.49	3.16	21.32	111
LSD $_{0,05}$	–	1.24	4.41	–	0.58	5.88	6.02	–	–	–	–	–
Average over the growing season												
Control	–	13.23	42.62	33.39	9.23	39.08	20.90	55.89	0.37	3.22	17.37	100
Polymyxobacterine	–	17.05	49.28	36.90	12.38	43.08	25.55	66.37	0.39	2.90	23.00	121

CFU – colony forming units;
MTSOM – coefficient of microbial transformation of soil organic matter;
TBI – total biological index;
LSD – least significant difference.

Impact of Polymyxobacterine on the number of phosphate-solubilizing bacteria in the soil root zone of sunflower plants



Effect of Polymyxobacterine on enzymatic activity of soil root zone of sunflower plants

Variant of experiment	Dehydrogenase, mg TPF / 100 g per 24 hours			Invertase, mg glucose / g per 24 hours			Phenol oxidase, mg 1,4-n-benzoquinone / 10 g per 1 hours		
	1	2	3	1	2	3	1	2	3
Control	**157.50**	**41.83**	**133.50**	**2.41**	**9.45**	**2.14**	**600.00**	**547.23**	**612.00**
Polymyxobacterine	**145.50**	**46.27**	**127.50**	**2.69**	**9.00**	**3.93**	**660.00**	**633.16**	**732.00**
$LSD_{0,05}$	14.86	4.02	9.68	0.19	0.52	0.34	29.20	26.14	34.97

1 – at the beginning of the growing season;

2 – in the middle of the growing season;

3 – at the end of the growing season;

TPF – triphenylformazan.

Effect Polymyxobacterine on the yield of sunflower

Without biological preparation	Application of Polymyxobacterine	LSD $_{0,05}$	Increase of yield	
			Centner · ha^{-1}	%
32.38	**41.67**	4.82	**9.29**	**28.69**

Use Polymyxobacterine in sunflower growing under conditions of organic farming increase harvest by 29%.

The ecological effect is to keep the environment clean and prevent soil pollution.

Correlation of biological indicators of podzolized chernozem with sunflower productivity

Biological indicators	Correlation coefficient (r)
Organic nitrogen assimilating bacteria	0,83
Mineral nitrogen assimilating bacteria	0,81
Actinomycetes	0,56
Organic phosphate-solubilizing bacteria	0,88
Mineral phosphate-solubilizing bacteria	0,65
Eutrophs	0,88
Oligotrophy index	-0,61
Mineralization index	-0,62
MTSOM	0,79
TBI	1,00
Dehydrogenase activity	0,47
Invertase activity	0,69
Polyphenol oxidase activity	0,83

Discussion

- The results of the study showed the feasibility of using Microhumin with moderate doses of mineral fertilizers. This helps to optimize plant nutrition, increase yields and minimizes the use of mineral fertilizers, as well as prevents soil and environmental pollution.

- Synthetic mineral fertilizers and chemical plant protection products cannot be used in organic farming. The use of Polymyxobacterine in an organic farming positively effects yields and solves the problem of fertilizing and protecting plants from pathogens.

Conclusions

- A positive effect of Microhumin on the soil biological indicators in the plants root zone soil and crops was revealed. The maximum effect on the yield was noted with the combined use of Microhumin and moderate doses of mineral fertilizers.
- Small-plot field experiment showed positive impact of seeds inoculation by Polymyxobacterine on the number of soil microflora and soil enzymatic activity in root zone of sunflower, buckwheat, maize plants. The degree of positive influence of treatment on the biological properties of soil and crop yield depended on the grown crop.
- According to the results obtained in the long-term stationary field experiment, a significant positive effect of biological preparation Polymyxobacterine for the number of microorganisms in the root zone of sunflower plants under organic farming condition was identified. On average, number of investigated micro-organisms increased by 21 %. Also soil enzymatic activities significantly increased: invertase by 12 – 84 %, dehidrogenase by 11 % phenol oxidase by 10 – 20 %. Sunflower yield was increased with use of Polymyxobacterine for 9.29 centner · ha^{-1} . The number of agronomically valuable phosphate-solubilizing bacteria significantly increased too.
- The economic effect of the use Polymyxobacterine in sunflower growing under conditions of organic farming is increasing harvest by 28.69 %. However, environmental effect is also valuable for the organic farming that means environmental cleanliness maintenance and preventing soil pollution.

Thank you !