

## Microbiological processes in rhizosphere of plants of peas at application of fertilizers and rhizohumin

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**The purpose.** To investigate influence of application of fertilizers and microbic preparation rhizohumin upon microbiological processes in rhizosphere of plants of peas and productivity of culture. **Methods.** Field researches, microbiological and biochemical methods of researches. **Results.** Favorable for development of agronomically valuable microorganisms at cultivation of peas on leached chernozem and formation of productivity of culture is after-action of dung, and also dose of mineral fertilizers, which do not exceed  $N_{60}P_{60}K_{60}$ . Use of rhizohumin is effective. Influence of biological product upon formation of productivity of peas in experiment is equivalent to action of fertilizers in the dose of  $N_{30}P_{30}K_{30}$ . **Conclusions.** Technique of cultivation of peas should necessarily include pre-seeding bacterization. At cultivation of the culture on leached chernozem the optimum backgrounds as to ecological-and-economic parameters are after-action of dung, application of fertilizers in dose which do not exceed  $N_{60}P_{60}K_{60}$ .

**Key words:** peas, nitrogen fixation, phosphate-mobilizing bacteria, fertilizers, chernozem.

Bacteria and micromyceta play an important essential and integral role in soil-microorganisms-plant system interactions. Roots are surrounded by microorganisms, which serve as trophic intermediary between soil and plants. Having a diverse and a complete set of microorganisms plants obtain maximum nutrition and, to a larger extent, protection from pathogens and, consequently, are capable of realizing their genetic yield potential [1-4].

Regulation of soil-microbial processes can be used for optimization of the production process of agricultural crops and ensure high quality output [5, 6]. One of the technological factors that can promote growth of useful soil microbiota is an application of modern microbial preparations [7-10].

The use of biological preparations in the technologies of legume crops cultivation is of the special importance, as the introduction of active rhizobia to a competitive agrocenosis can stimulate formation and functioning of high nitrogen fixing plant-bacterial symbioses and significantly affect nitrogen metabolism of plants [5].

However, the positive effect of microbial preparations in agricultural production is often leveled due to the ignorance of microorganism's interaction peculiarities with plants. In particular, the presence of excess mineral nitrogen in soil adversely affects the functioning of the legume-rhizobial symbiosis. At the same time, the use of fertilizers within their physiological expediency can stimulate nitrogen fixation activity as well as other microbial processes in soil important for plants [11]. The same is also true to the phosphorous mobilizing microorganisms.

The **purpose of present research** was to investigate the effect of fertilizers and pre-sowing seeds inoculation on microbiological processes in rhizosphere soil of pea plants and crop yield.

**Methods.** The study was conducted during 2013 – 2015 years on leached chernozem ( $\text{pH}_{\text{salt}} - 5.2$ ; humus content – 3.01%, easily hydrolyzed nitrogen – 109 mg/kg, mobile forms of phosphate ( $\text{P}_2\text{O}_5$ ) – 168 mg/kg (by Kirsanov), exchangeable potassium content ( $\text{K}_2\text{O}$ ) (by Kirsanov) – 58 mg/kg of soil) of experimental fields of Institute of Agricultural Microbiology and Agricultural Production NAAS (stationary field experiment with short crop rotation: potato – barley – peas – winter wheat) under the different fertilization conditions. Pea cultivation (var. Deviz) was conducted in two blocks – without inoculation and under the use of microbial preparation Rhizohumin. Fertilization system of crops was as follows:  $\text{N}_{30}\text{P}_{30}\text{K}_{30}$ ,  $\text{N}_{60}\text{P}_{60}\text{K}_{60}$ ,  $\text{N}_{90}\text{P}_{90}\text{K}_{90}$ , second year aftereffect of manure (40 t/ha) and organic-mineral fertilizer (aftereffect of manure (40 t/ha) +  $\text{N}_{30}\text{P}_{30}\text{K}_{30}$ ). Organic fertilizers were applied prior to potatoes cultivation. Research plots area was 86.4 m<sup>2</sup> each (7.2 x 12.0), repeated four times.

Seeds inoculation was performed according to the SOU 01.11-37-782 [12] for the use of microbial preparation Rhizohumin (TU 24.1-00497360-007). Rhizohumin – is a preparation of a complex action, that besides the active strain of pea nodulating bacteria has physiologically active substances of natural origin and bacterial associations, including phosphorous mobilizing microorganisms.

The number and mass of root nodules of pea plants was calculated in dynamics. The activity of symbiotic nitrogen fixation was studied using acetylene method [13] in closed chambers [14]. Plastic 10 l buckets equipped with the rubber stopper for gases separation were used, mounted into the soil to a depth of 5 cm. “Water plug” was made around the chambers for better system isolation and prevention of losses of gases, accumulated in the chambers. Vessel with water and calcium carbide added prior to exposition was put in the middle of the chamber. Exposure time – 3 hours.

Gas samples were analyzed on a gas chromatograph “Chrom-5” with a flame ionization detector and sorption steel columns filled with sorbent ParapakQ 60-80 mesh. Thermostat temperature was 40 °C. Gases flow used: hydrogen – 15 cm<sup>3</sup>/min, nitrogen – 100 cm<sup>3</sup>/min, air – 500 cm<sup>3</sup>/min.

The number of bacteria capable of dissolving mineral phosphorus and hydrolysis of organophosphates in soil was determined on Muromtsev nutrient medium, phosphatase activity – by Geller and Ginsburg method, phosphorus content in grain – by Denizhe method modified by Buvat'e [15]. The protein content in grain was determined according to DSTU 7169 [16]. Experiments design and harvest record was performed according to Dospikhov [17]. Statistical analysis of the results was performed using analysis of variance [18] and a computer software (Microsoft Office Excel 2003 – 2007).

**Results.** Study of pea nodulation activity on different fertilization background indicates on growth of nitrogen fixing nodules in variants with fertilizer doses not exceeding  $\text{N}_{60}\text{P}_{60}\text{K}_{60}$ . High fertilizer rates, manure aftereffect and use of organic-mineral fertilizers had no positive effect nodules formation. Under the influence of Rhizohumin the nodulation activity had increased in almost all the studied variants with the exception of the highest rate of fertilizers used (Table 1). In this variant nodules growth was observed only during the pods formation stage.

Nodules mass was also the largest in the variants with low fertilizer doses (Table 1). Its increase was observed in the variants with the highest fertilizers doses only by the end of the growth period. Use of Rhizohumin was the most influential factor affecting the formation of mass nodules.

Observed peculiarities of nitrogen fixation symbiotic system formation was also confirmed during the other of study years.

Determination of symbiotic nitrogen fixation dynamics had also shown a clear dependence of performance characteristic upon the fertilization (Fig. 1). Thus, during the budding and flowering stages the highest nitrogen fixation activity was observed in variants with fertilizer doses not exceeding  $\text{N}_{60}\text{P}_{60}\text{K}_{60}$ . Application of biological preparation on low and medium fertilizer backgrounds had stimulated nitrogen fixation activity even at greater extent.

**1. Formation of symbiotic nitrogen fixation apparatus on pea plants under the influence of fertilizers and Rhizohumin, 2014.**

Variants	Number of nodules, pcs./plant			Mass of nodules, mg/plant		
	Budding	Flowering	Pod formation	Budding	Flowerin g	Pod formation
<i>without inoculation</i>						
w/o fertilizers, control	19,0	24,6	29,3	131,00	176,33	190,67
Manure, 40 t/ha (aftereffect)	21,2	22,3	32,6	125,67	140,67	224,67
N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	25,0	32,0	40,0	174,67	205,67	311,00
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	24,3	27,7	38,0	148,67	188,00	301,67
N <sub>90</sub> P <sub>90</sub> K <sub>90</sub>	19,3	22,6	37,7	120,00	148,67	298,33
Manure, 40 t/ha (aftereffect) + N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	22,0	25,7	32,6	131,33	173,67	256,00
<i>inoculation with Rhizohumin</i>						
w/o fertilizers, control	30,0	35,7	37,3	199,3	237,00	286,33
Manure, 40 t/ha (aftereffect)	22,3	28,3	39,0	132,00	184,67	275,00
N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	32,7	37,5	43,0	246,67	314,00	320,67
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	30,3	34,0	41,3	207,67	281,00	311,67
N <sub>90</sub> P <sub>90</sub> K <sub>90</sub>	26,3	24,3	42,0	161,00	176,00	317,67
Manure, 40 t/ha (aftereffect) + N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	27,7	29,0	38,0	164,00	214,33	293,67
LSD <sub>05</sub> experiment	5,3	4,0	5,2	18,0	24,3	22,0
fertilizers	3,7	2,1	2,6	9,2	13,3	12,0
biopreparation and interaction	2,6	2,0	2,6	9,0	12,6	11,0

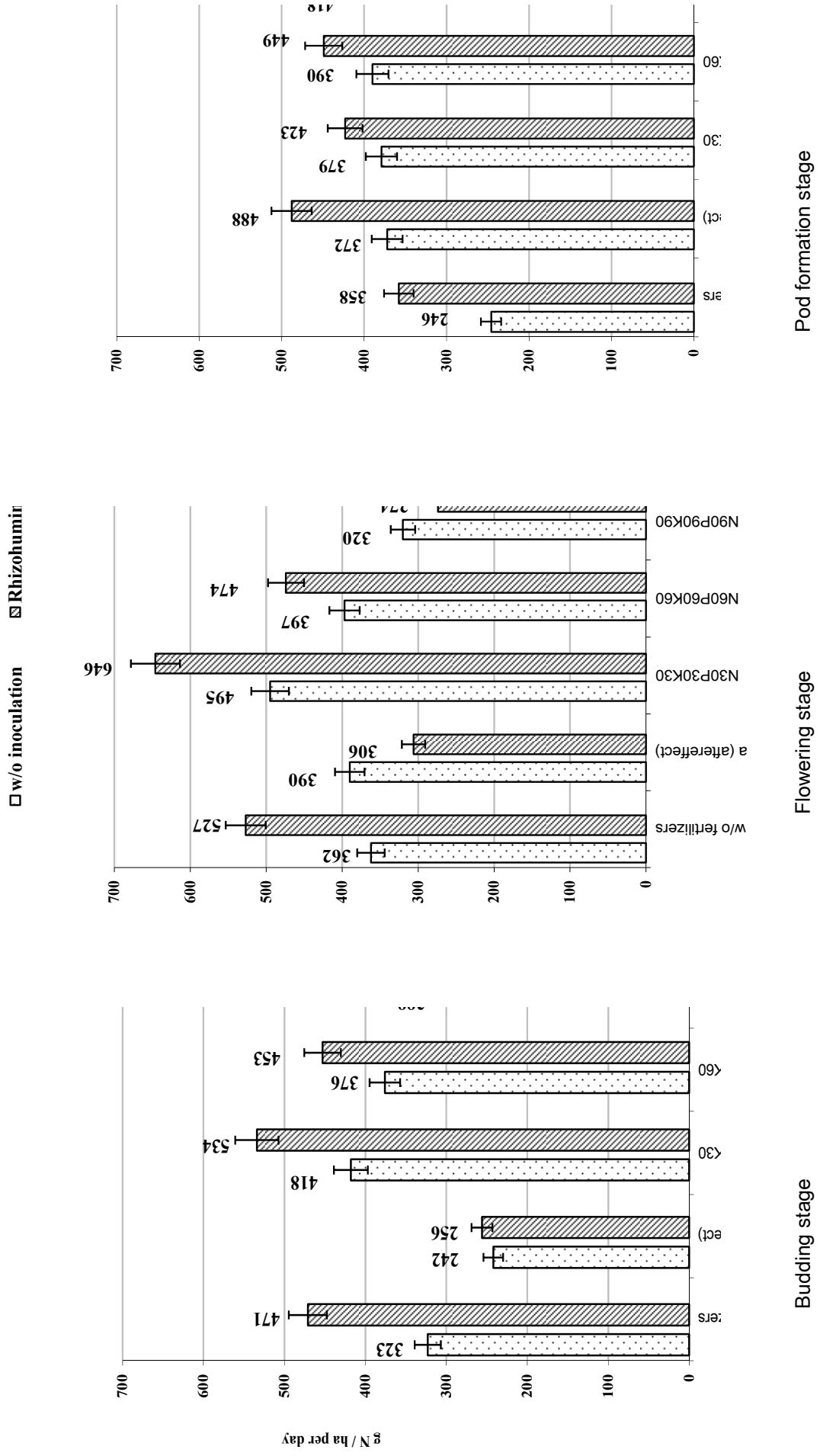


Figure 1. Nitrogen fixation dynamics in peas crop systems under the influence of fertilizers and biological preparation, 2014.

The decline of nitrogen fixation activity was observed during the initial growth stages in variants with the aftereffect of manure, organic-mineral fertilizer and the highest fertilizer dose comparing to control. Its recovery was observed during the flowering stage in the variants with manure aftereffect. By the end of the growing season all variants with fertilizer application had shown higher nitrogen fixation activity than in control. At this, Rhizohumin usually enhances process activity. Similar features of fertilizer and pre-sowing seeds inoculation action were observed during the other study years, thus indicating on the possibility of pea cultivation on leached chernozem soils with fertilizers doses that do not exceed  $N_{60}P_{60}K_{60}$  and use of biological preparation Rhizohumin, which provide optimal conditions for the formation and functioning of legume-rhizobial nitrogen fixing symbiosis.

The analysis of total nitrogen fixation productivity in pea plants during 2013 – 2015 demonstrate the positive effect of inoculation especially on low and medium fertilizer backgrounds (Table 2). Without fertilizers Rhizohumin enhances nitrogen fixation productivity by 36.6% compared to the control. However, the highest indices were observed in the application of  $N_{30}P_{30}K_{30}$  dose of fertilizer. Slightly lower, but still higher than the ones in the variants without fertilizers, was nitrogen accumulation values in the variant with Rhizohumin application on  $N_{60}P_{60}K_{60}$  background.

Application of Rhizohumin on optimal fertilizer's background had stimulated nitrogen fixation activity in 1.2 – 1.3 times.

The study of microorganisms' populations capable of dissolving mineral forms of phosphates had revealed that fertilizers stimulate their growth in all variants with the exception for a high doses (Fig. 2). In all variants, application of Rhizohumin had boosted the number of these microorganisms which is largely appears due to the presence in the preparation of phosphorus mobilizing bacteria in addition to rhizobia.

**2. Total nitrogen fixation productivity in peas crop systems under the influence of fertilizers and Rhizohumin.**

Variant	Nitrogen, kg/ha							
	2013		2014		2015		Average for three years	
	w/o inoculation	Rhizohumin	w/o inoculation	Rhizohu min	w/o inoculation	Rhizohu min	w/o inoculation	Rhizohu min
w/o fertilizers, control	25,83	35,19	28,20	39,17	27,77	37,35	27,26	37,24
Manure, 40 t/ha (aftereffect)	39,21	37,62	28,17	29,57	26,08	32,03	31,15	33,07
N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	39,00	46,80	36,15	45,72	36,82	46,22	37,32	46,25
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	39,60	44,91	33,55	39,40	33,80	40,54	35,65	41,61
N <sub>90</sub> P <sub>90</sub> K <sub>90</sub>	29,19	36,57	29,07	30,45	32,03	32,91	30,10	33,31
Manure, 40 t/ha (aftereffect) + N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	32,76	34,77	31,32	26,17	27,06	33,9	30,38	31,61

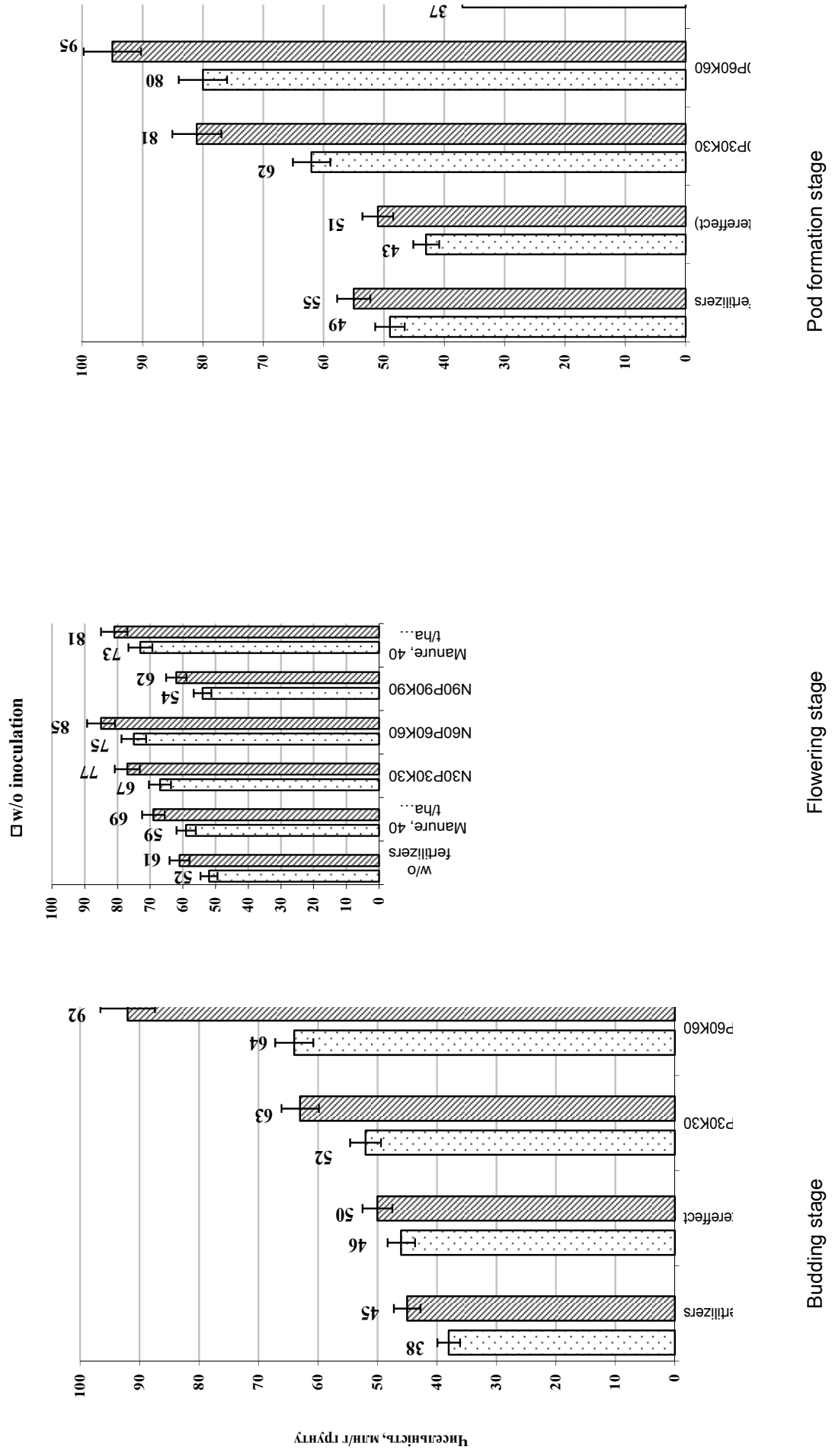
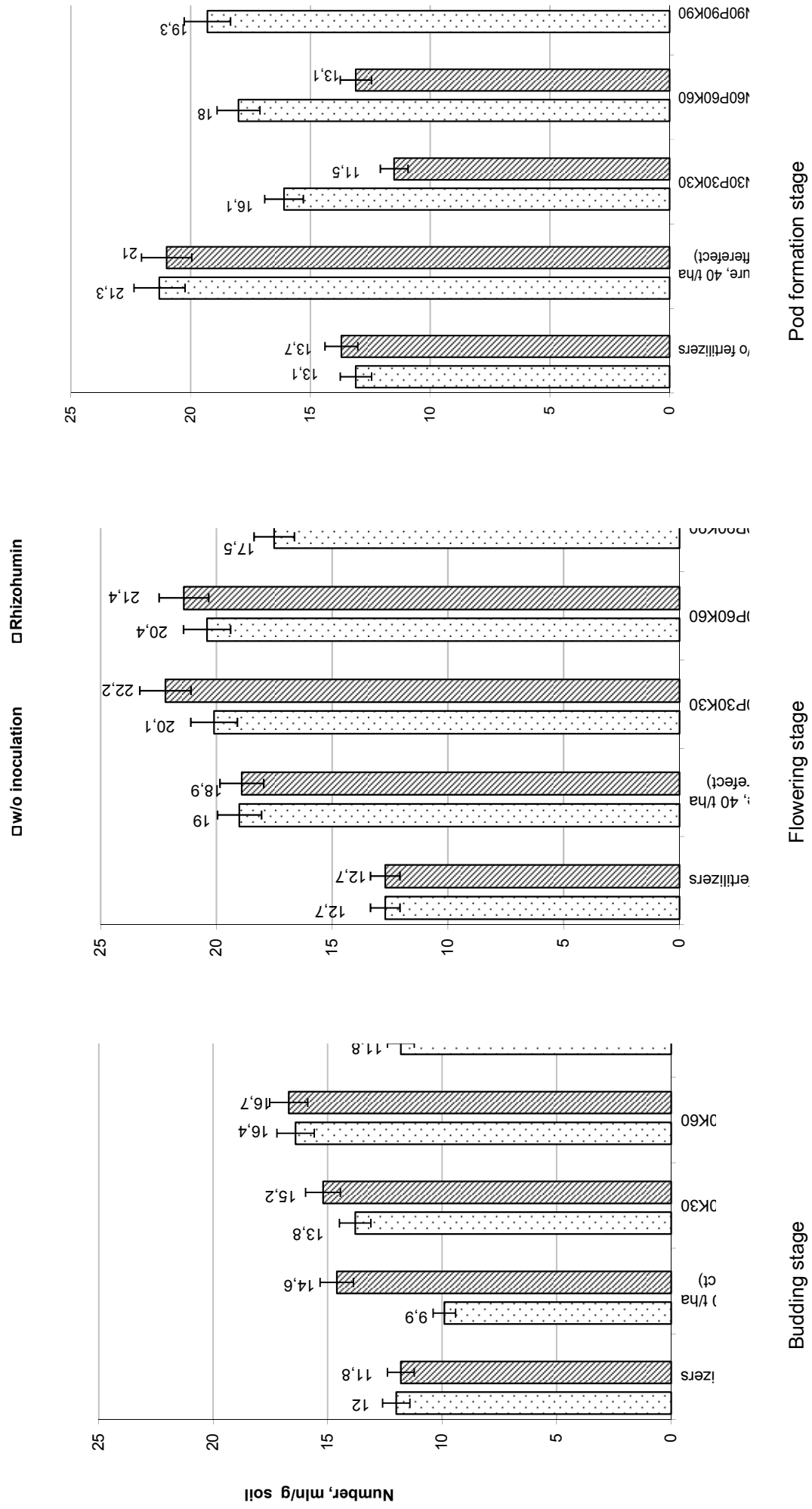


Fig. 2. The influence of fertilizers and inoculation on the number of microorganisms that dissolve mineral phosphates in rhizosphere soil of pea plants

Under the use of biological preparation the significant growth of microorganisms that hydrolyze organophosphates on all studied fertilizer background was observed. Arguably, it has an indirect effect – through the activation of plants growth and development processes and increase of plant metabolites volumes in root zone (Fig. 3). The clear decrease of studied bacteria number in variants with Rhizohumin, with the exception of variant with high dose of mineral fertilizers) was noticed only during the pod formation stage. We associate this to the fact that inoculated plants pass certain developing stages faster comparing to not inoculated ones. This assumption is enforced by a slight increase in the number of bacteria in the variant  $N_{90}P_{90}K_{90}$ . Intensive mineral nutrition of plants using high fertilizer doses extend vegetation period, which ensures positive effect of biological preparation on the development of phosphorus mobilizing bacteria in given case.

Observed features were confirmed in general by studies conducted in other years, and the results of phosphatase activity determination in the rhizosphere soil of pea plants, which is closely related to the number of phosphorus mobilizing bacteria capable of hydrolysis of organic forms of phosphates.

Analysis of fertilizers' effect on pea yield formation during 2013 – 2015 had revealed only a slight crop productivity increase on the background of manure aftereffect – 0.3 t/ha (Table 3). However, Rhizohumin application had doubled it to 0.6 t/ha or 27.3%. Given the low production cost under these conditions appeal of given fertilizer system is obvious.



**Fig. 3. The influence of fertilizers and inoculation on the number of microorganisms that dissolve organic phosphates in rhizosphere soil of pea plants**

**Table 3. The influence of fertilizers and Rhizohumin on yield of pea plants**

Variant	Yield, t/ha (average for three years)	Increment			
		to fertilizers *		to inoculation	
		t/ha	%	t/ha	%
<i>w/o inoculation</i>					
w/o fertilizers, control	2,2	-	-	-	-
Manure, 40 t/ha (aftereffect)	2,5	0,3	13,6	-	-
N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	2,7	0,5	22,7	-	-
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	3,1	0,9	40,9	-	-
N <sub>90</sub> P <sub>90</sub> K <sub>90</sub>	3,2	1,0	45,5	-	-
Manure, 40 t/ha (aftereffect) + N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	3,0	0,8	36,4	-	-
<i>inoculation with Rhizohumin</i>					
w/o fertilizers	2,5	-	-	0,3	13,6
Manure, 40 t/ha (aftereffect)	2,8	0,6*	27,3	0,3	12,0
N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	3,2	1,0*	45,5	0,5	18,5
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	3,5	1,3*	59,1	0,4	12,9
N <sub>90</sub> P <sub>90</sub> K <sub>90</sub>	3,6	1,4*	63,6	0,4	12,5
Manure, 40 t/ha (aftereffect) + N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	3,4	1,2*	54,5	0,4	13,3
LSD <sub>05</sub> experiment	0,29				
fertilizers	0,18				
Inoculation and interaction	0,13				

\*) including interaction with the preparation

As the agrochemical load increases the pea yield was growing although the return by each subsequent dose of fertilizers in the experiment was lower. Rhizohumin substantially increases the efficiency of fertilizers application which was most evident on low and medium fertilizer doses background – N<sub>30</sub>P<sub>30</sub>K<sub>30</sub> and N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>. The effect of biological preparation on crop productivity in these variants was equal to the impact of fertilizer in dose not less than N<sub>30</sub>P<sub>30</sub>K<sub>30</sub>.

Pea seed inoculation was also beneficial to the grain quality influencing, in particular, protein and phosphorus content (Table 4).

#### 4. The influence of fertilizers and inoculation on the protein and phosphorous content in pea grain, 2014

Variant	P <sub>2</sub> O <sub>5</sub> , %	Protein, %
<i>w/o inoculation</i>		
w/o fertilizers, control	1,26±0,02	22,2±0,3
Manure, 40 t/ha (aftereffect)	1,31±0,02	23,6±0,3
N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	1,20±0,01	23,5±0,3
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	1,33±0,02	24,3±0,3
N <sub>90</sub> P <sub>90</sub> K <sub>90</sub>	1,29±0,01	23,3±0,4
Manure, 40 t/ha (aftereffect) + N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	1,34±0,02	23,9±0,3
<i>Inoculation with Rhizohumin</i>		
w/o fertilizers,	1,29±0,02	22,4±0,2
Manure, 40 t/ha (aftereffect)	1,45±0,02	23,9±0,3
N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	1,46±0,02	24,0±0,5
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	1,44±0,02	24,8±0,5
N <sub>90</sub> P <sub>90</sub> K <sub>90</sub>	1,66±0,02	24,0±0,2
Manure, 40 t/ha (aftereffect) + N <sub>30</sub> P <sub>30</sub> K <sub>30</sub>	1,58±0,02	24,4±0,3

Relying on the analysis of yield productivity and grain quality only, the economic attractiveness of the investigated factors can be determined. Nowadays, however, it is vital to establish the safety standards for agrochemical use, including fertilizers application. Given that the growth of microorganisms in the root zone of plants as well as the intensity of their relevant processes can be used as an indicator of environmental feasibility of various agricultural practices (e.g. nitrogen fixing bacteria do not bind atmospheric nitrogen under the conditions of excess mineral nitrogen present in the environment [18]) the environmental and economic practicability of growing peas on leached chernozem on the fertilizer background that do not exceed N<sub>60</sub>P<sub>60</sub>K<sub>60</sub> is apparent. Undoubtedly beneficial in this connection is crop cultivation on the manure aftereffect background combined with Rhizohumin application. Under these conditions the relatively low yield increase is leveled by the low production costs (practically elimination of fertilizers and their application costs). High fertilizers doses restrict nitrogen fixation potential, negatively affect the development of phosphorus mobilizing microorganisms and provide relatively low yield increase compared to the low and medium fertilizer doses.

#### Conclusions.

Pre-sowing seeds inoculation of pea plants with the biological preparation Rhizohumin plays an important role in optimization of vital microbial processes in the soil at pea cultivation on leached chernozem. Crop cultivation was proved to be the optimal under the fertilizer use in doses that do not exceed N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>, as well as on manure aftereffect. The combination of studied fertilizer systems with Rhizohumin application had ensured a significantly higher increase of the production efficiency.

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