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INFLUENCE OF BIOLOGICAL PRODUCTS ON THE MICROBIOM SOIL IN THE RHIZOSPHERE OF *GLYCINE MAX (L.) MERR.*

O.O. Alieksieiev[1], V.F. Patyka[2]
[1] Assistant, [2] Doctor of Biological Sciences, Professor of Department of Phytopathogenic Bacteria
[1] Vinnytsia National Agrarian University,
[2] Institute of Microbiology and Virology of NASU named after Acad. D.K. Zabolotny (Kyiv), Ukraine

Abstract. *Influence of biological preparations on the micro biome of soil of soybean rhizosphere is studied in the article. The description of the number of different ecological and trophic groups of soil microorganisms was carried out. Special attention was given to the study of influence of biological preparations, in particular, rhizobofit on ammonifying and nitrifying soil ability. The study on determining the typical and predominant kinds of spore bacteria in rhizosphere and spacing by rhizobofit action in relation to mineral fertilizers is conducted. Yield capacity of soybean varieties Horlytsia and KyVin on control as well as yield increase due to rhizobofit and rhizobofit in combination with mineral fertilizer is presented.*

Keywords: *soybean, rhizosphere, microbiocoenosis, saprofite, bacilli, oligonitrophil, streptomyces, fungi.*

Functional qualities, number and structure of microbial groups are susceptible to the impact of unfavourable factors. Revealing principal regularities of the influence of environment conditions on metabolism of microorganisms is a scientific basis for the development of the new biotechnologies in agrarian production, providing great opportunities for the direct synthesis of cellular macromolecules and obtaining target products of microbial process. It should be mentioned that ecological functions of microbial groups are so essential and unique that any changes within them may affect the whole ecosystem [3]. It is noted that at present populations of microorganisms concentrated in the soil profile are the indicators of ecological state and soil fertility [8]. Therefore, measures on improvement of agriculture efficiency and recovery of soil fertility should necessarily be connected with microorganisms' activity.

Thus, dissemination of certain groups of microorganisms assimilating mineral forms of nitrogen in the soil tells about active course of processes of mineralization of organic substances. Domination of facultative and anaerobic varieties in the soil may reveal the worsening of soil aeration conditions. Strengthening fungi-static potential of soil is a favourable condition for reproduction of bacterial micro biota. Availability of spore bacteria reveals the soil being provided with organic source of nitrogen and the activity of mineralizing processes [2, 9].

Therefore, for studying quantitative and qualitative composition of microbial rhizosphere groups and for determining the properties of predominant varieties legume crop *Glycine max (L.) Merr.* was used. Studying the problem of microbial environment creation and investigating the influence of introduced microorganisms on the quantitative and qualitative composition of microbiocoenosis of soybean will provide wider use of bio preparations while growing legumes.

Soil micro flora, which was formed under soybean crops, served as an ecosystem indicator of succession processes, which is a rather actual issue. Correlation of ecologic and trophic groups of soil microorganisms changes depending on the plant. Thus, the number of amonificators decreased from 18.7•10^6 to 4.3•10^6 with the change of a crop (soybean – lupine – wheat – rape), a similar regularity was observed for oligotrophs and pedotrophs (figure1).

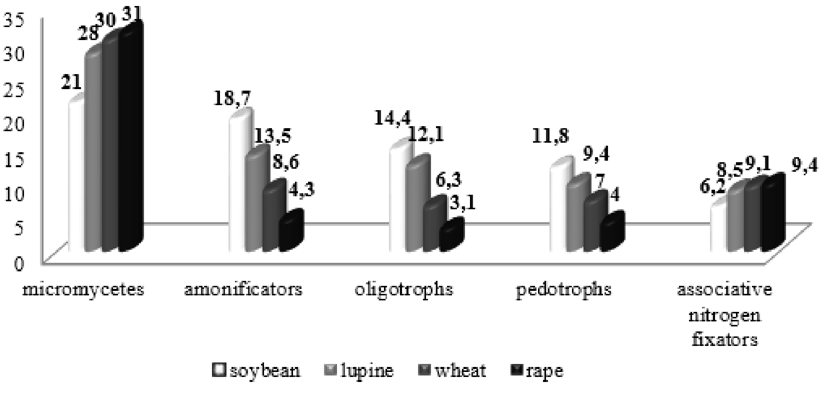

Fig. 1. The average number of ecologic and trophic groups of microorganisms in the soil under soybean crops (CFU per 1 g of abs. dry soil) in 2013-2015

In figure 1 we see that the content of amonificators under given crop was 18.7 million of CFU /g of abs. dry soil, which was 4.4 times more than under rape growing. Given indicators reflect actual soil enrichment with organic substance of plant origin, as well as provide additional amount of ammonium nitrogen due to its fixation from the air. The corresponding changes in number were observed in case with associative nitrogen fixators that used mineral nitrogen for self-nutrition. The maximal number of these microorganisms in soil was marked under growing wheat and rape and amounted 9.1 and 9.4 of CFU /g of abs. dry soil accordingly. Regarding micromycetes, it should be noted that fluctuations in their numbers were not as significant as of the bacterial flora, but in agrocenoses of wheat, lupine, rape, it was higher than in the soil under soybean.

In the course of the further studies, the essential changes were revealed also in the dynamics of microflora number, which provided processes of incoming and decomposition of organic substance. Thus, the group of saprophytic microorganisms – bacilli (table 1), which was the most numerous in the soil environment differed by joint application of mineral fertilizers and rhizobofit during the phase of soybean flowering, though the number of oligonitrofil bacteria under the same conditions reduced significantly. The difference in experimental variants is not essential in the case of streptomycetes and fungi.

Table 1

Dynamics of the number of microorganisms in grey medium loam soils under growing soybean with the application of various types of fertilizers

Variant	Control	$N_{30}P_{30}K_{30}$	*Bradyrhizobium japonicum* M8 (rhizobofit)
Bacilli (million/g)			
May	0.88	1.91	3.20
June	1.20	2.95	5.90
July	2.09	5.15	9.23
August	1.07	3.06	6.10
September	0.96	2.02	4.06
$LSD_{0,5}$	0.41	0.62	1.01
Oligonitrofils (million/g)			
May	5.35	8.19	9.98
June	2.10	4.01	5.45
July	1.89	3.75	5.15
August	1.71	2.13	4.05
September	0.60	2.10	4.23
$LSD_{0,5}$	1.32	2.05	1.97
Fungi (thousand/g)			
May	0.07	0.09	3.90
June	2.07	3.98	5.03
July	1.92	3.78	5.18
August	1.48	2.15	4.05
September	1.45	2.20	4.53
$LSD_{0,5}$	0.44	0.97	0.38

Having applied *Bradyrhizobium japonicum* M8 (rhizobofit) in growing soybean, the positive influence of the action of the given component on the amonifying and nitrifying process of microorganisms in the soil environment was detected. Thus, amonifying soil ability increased up to 282 mg NH_3/100 g of soil, and nitrifying – up to 62 mg NO_3/100 g of soil in comparison with the control, which can be seen in the figures 2, 3.

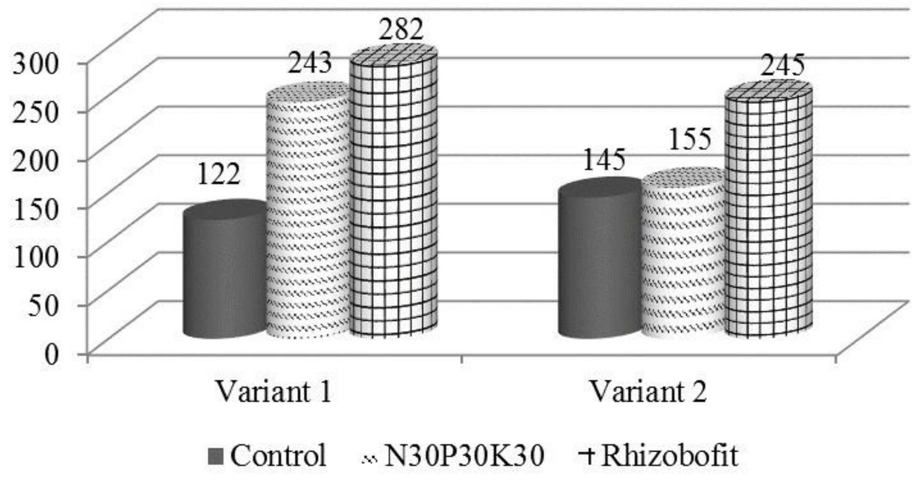

Fig. 2. Amonifying activity of grey forest medium loam soil under growing soybean of Horlytsia variety using various types of fertilizers, NH_3 mg/100 g of soil (1 – variant without fertilizers; 2 – variant $N_{30}P_{30}K_{30}$ + rhizobofit)

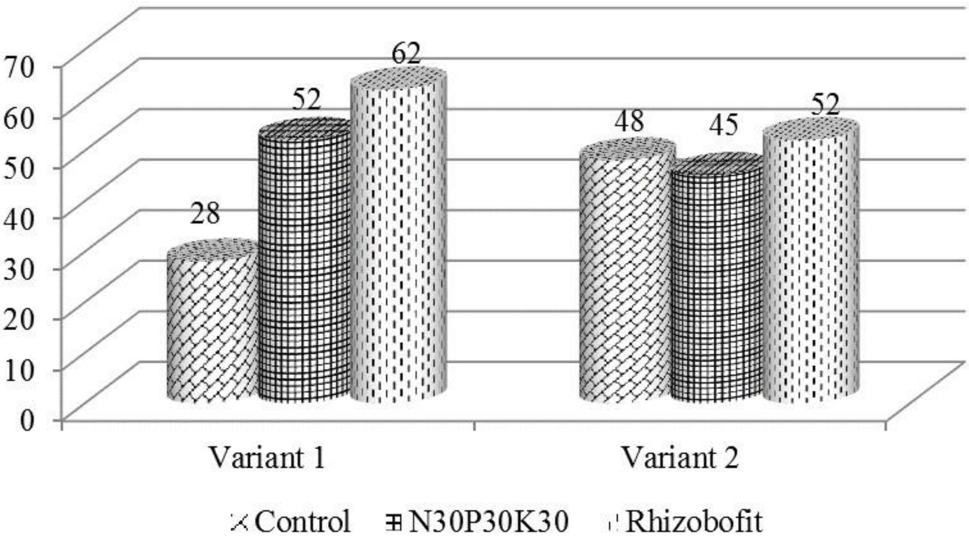

Fig. 3. Nitrifying activity of grey forest medium loam soil under growing soybean of Horlytsia variety using various types of fertilizers, NO_3 mg/100 g of soil (1 – variant without fertilizers; 2 – variant $N_{30}P_{30}K_{30}$ + rhizobofit)

The next stage of the research was to determine the typical and predominant varieties of spore bacteria in soybean rhizosphere with the use of different kinds of fertilizers.

During the study of typical and predominant kinds of spore bacteria in the basal part of soybean that is in rhizosphere, which was influenced by soil microorganisms, the following tendency was observed. Thus, in the control variant without fertilizers frequency of occurrence of varieties (figure 4) *B. macerans*, *B. cereus*, *B. subtilis* were 90 %, 90 % and 70 % accordingly, and a share of varieties concentrated in rhizosphere was 64 %, 18 % and 8 %.

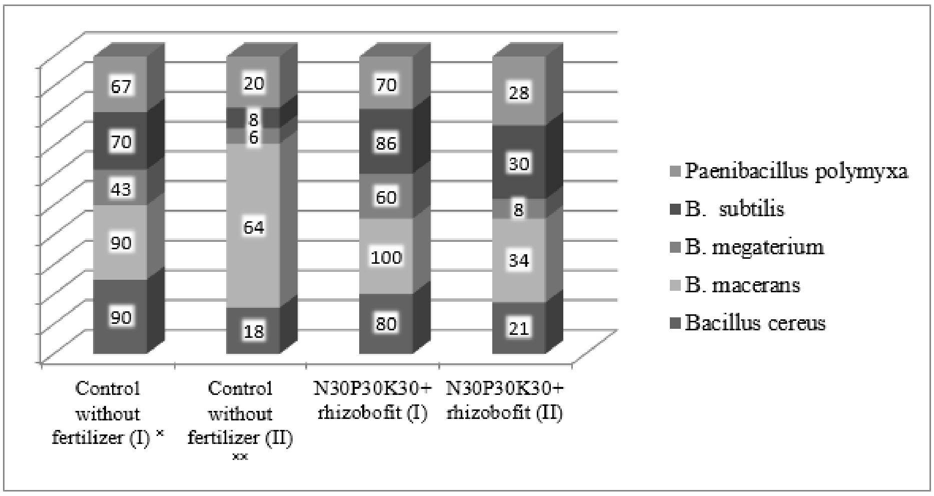

*Fig. 4. Typical and predominant kinds of spore bacteria in soybean rhizosphere with the use of different kinds of fertilizers (Note: * – frequency of occurrence of varieties, %; ** – share of concentration type, %)*

In the next stage of the research, it was expected to carry out the analysis of the variant $N_{30}P_{30}K_{30}$ + rhizobofit in relation to the interaction of the given substances with microorganisms. During the observations, it was noted that the frequency of occurrence of varieties *B. macerans, B. subtilis, B. cereus was* 100 %, 86 %, 80 % accordingly, and the share of concentration type of microorganisms in rhizosphere was 34 %, 30 % and 21 %. Comparison of a control variant with the variant with mineral fertilizer + rhizobofit showed that microorganisms of bacilli class of variety *B. macerans* and *B. subtilis* increased their range of distribution frequency by 10 % and 16 % accordingly. Thus, it should be concluded that the interaction of microorganisms and biological inoculant on the basis of microorganisms of the strain *Bradyrhizobium japonicum* M8 contributed each other and enriched soil micro flora, in particular, basal part of soybean, which will contribute further yield growth of the given crop.

During the study of rhizosphere of basal part of soybean it was found out that there is a range of other representatives of bacilli class, concentrated in soil layer of the researched territory in particular in the spacing part (figure 5).

Thus, in the control variant without fertilizers, the rather high frequency and the share of the occurrence of the variety of bacilli class representatives was also observed. In particular, frequency of the occurrence of *B. macerans* variety was 90 %, as in rhizosphere, but the share of the occurrence of the variety appeared to be lower in comparison with rhizosphere accumulation of microorganisms and totalled 45 %. However, it is noted that *B. cereus* + *B. mycoides* also have rather high frequency of variety occurrence – 90 % and a share of the variety 19 %.

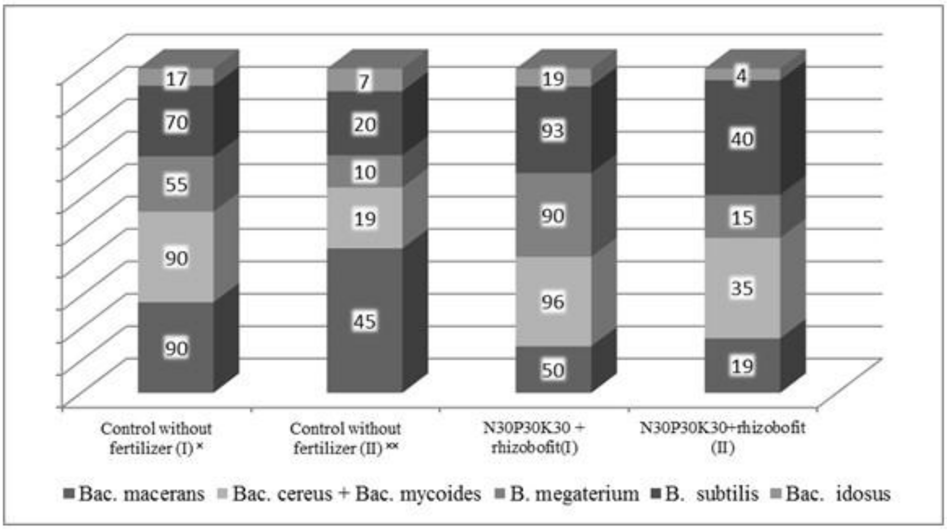

*Fig. 5. Typical and predominant kinds of spore bacteria in soybean spacing with the use of different types of fertilizers (Note: * – frequency of occurrence of varieties, %; ** – share of concentration type, %)*

Stable indicator of 70 % was observed also in *B. subtilis* variety both in rhizosphere part of the soil and in spacing, though the share of the variety in spacing was 12 % higher than in rhizosphere.

During the analysis of the obtained data on application of fertilizers $N_{30}P_{30}K_{30}$ + rhizobofit in spacing it was found out that the frequency of the occurrence of the variety and the share of variety of representative of *B. macerans* variety was to some extent depressed and totalled 50 % and 19 % accordingly. However, the following representatives, in particular, *B. cereus* + *B. mycoides*, *B. subtilis* appeared to be 96% and 93% accordingly.

Therefore, due to the analysed data it is studied that the increase of spore bacteria in soil in particular microorganisms of bacilli class of *B. macerans* and *B. subtilis* varieties in the variant of rhizobofit + mineral fertilizers indicates that mobilization processes are significantly more intensive under the use of organic fertilizers than in variants without fertilizers.

From the presented data (table 2) it is noticed that the growth of yield of virus resistant variety of soybean Horlytsia in the variant with rhizobofit in comparison with the variant without inoculation was 0.65 t/ha accordingly.

Table 2

Yield capacity of soybean of Horlytsia variety depending on fertilizing (average in 2013–2015)

Variant	Grain yield capacity, t/ha	Yield increase in relation to control	
		t/ha	%
Control	1.74	-	-
$N_{30}P_{30}K_{30}$	2.46	0.72	29.3
Control + *B. japonicum* M8 (rhizobofit)	2.39	0.65	27.2
$N_{30}P_{30}K_{30}$+ *B. japonicum* M8 (rhizobofit)	2.58	0.84	32.6

Application of rhizobofit also positively influenced the soybean yield capacity in relation to mineral fertilizers ($N_{30}P_{30}K_{30,}$ – increase 0,72t/ha or 29.3 %) with the yield increase less than by 2.1 %. An essential indicator of yield capacity unlike control 1.74 t/ha was obtained by combination of $N_{30}P_{30}K_{30}$+ *Bradyrhizobium japonicum* M8 (rhizobofit), which amounted 2.58 t/ha or 32.6 % of yield increase. The same situation was observed with the variety of soybean KyVin (table 3) under obtaining grain yield capacity.

Table 3

Yield capacity of soybean of KyVin variety depending on fertilizing during (average in 2013–2015)

Variant	Grain yield capacity, t/ha	Yield increase in relation to control	
		t/ha	%
Control	1.83	-	-
$N_{30}P_{30}K_{30}$	2.32	0.49	21.1
Control + *B.japonicum* M8 (rhizobofit)	2.41	0.58	24
$N_{30}P_{30}K_{30}$+ *B. japonicum* M8 (rhizobofit)	2.62	0.79	30.1

Thus, in control variant yield capacity amounted 1.83 t/ha was obtained, and yield increase by 24 % more was obtained in variant with the application of inoculant rhizobofit (*Bradyrhizobium japonicum* M8) which in general yield capacity amounted 2.41 t/ha. Though the best result was obtained in the variant with the combination of mineral fertilizer $N_{30}P_{30}K_{30}$ + *Bradyrhizobium japonicum* M8 (rhizobofit) and amounted 30.1 %, which is equivalent to 0.79 t/ha of yield increase to the control, and the control in its turn amounted 1.83 t/ha.

Thus, in the research the analysis of soil micro flora under soybean crops, which played the role of ecosystem indicator of succession processes, was carried out. It was determined that the best development of microorganisms amounted as follows: amonificators – 18.7 million CFU/g of abs. dry soil, oligotrophs –14.4 million CFU/g of abs. dry soil and pedotrophs –11.8 million CFU/g of abs. dry soil provide actual enrichment of soil with organic substance as well as provide additional fixation of nitrogen from air.

Having observed the dynamics of the development of microorganism's number of soybean rhizosphere under the action of biological preparation – rhizobofit and mineral fertilizers the following results were revealed:

1. Positive influence of application of rhizobofit on the improvement of micro flora is found out, in particular, amonifying ability of soil increased by 131 %, and nitrifying by 121 % in comparison with control.
2. The analysis of typical and predominant kinds of spore bacteria in rhizosphere and soil spacing was carried out, as a result of which the increase of representatives of bacilli class *B. cereus + B. mycoides* – 96 %, *B. megaterium* – 90 %, *B. subtilis* – 93 % on the account of rhizobofit + $N_{30}P_{30}K_{30}$ was observed.
3. Growth of yield of the soybean variety Horlytsia was obtained – by 27.2 % and KyVin variety – by 24 % more with rhizobofit unlike the control variant.

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ВЛИЯНИЕ БИОПРЕПАРАТОВ НА МИКРОБИОМ ПОЧВЫ РИЗОСФЕРЫ *GLYCINE MAX (L.) MERR.*

А.А. Алексеев[1], В.Ф. Патыка[2]

[1] ассистент, [2] доктор биологических наук, профессор, Отдел фитопатогенных бактерий

[1] Винницкий национальный аграрный университет,

[2] Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины (Киев), Украина

Аннотация. *В статье исследуется влияние биологических препаратов на микробиом почвы ризосферы сои. Было проведено описание численности различных эколого-трофических групп почвенных микроорганизмов. Особое внимание было уделено изучению влияния биологических препаратов, а именно, ризобофита на амонифицирующую и нитрифицирующую способность почвы. Проведено исследование по определению типовых и доминирующих видов споровых бактерий в ризосфере и междурядье при воздействии ризобофита на фоне минеральных удобрений. Показана урожайность сои сортов Горлица и КиВин на контроле, а также прирост урожая за счет ризобофита и ризобофита в сочетании с минеральным удобрением.*

Ключевые слова: *соя, ризосфера, микробоценоз, сапрофиты, бациллы, олигонитрофилы, стрептомицеты, грибы.*