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DEVELOPMENT OF NATURAL SCIENCES IN COUNTRIES OF THE EUROPEAN UNION TAKING INTO ACCOUNT THE CHALLENGES OF XXI CENTURY

Collective monograph

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THE EFFICIENCY OF COMBINED USE OF INOCULATION AND EM-TECHNOLOGIES IN THE CULTIVATION OF LEGUMES

Kononchuk O. B., Pyda S. V.

INTRODUCTION

Negative features of modern intensive agriculture include contamination of the natural environment, increased energy consumption, increased doses of fertilizers and other chemical substances used for agriculture, which no longer provides an effective increase in yield. In order to prevent a tense ecological situation, a new strategy of management, based on the principles of biological protection and plant nutrition, is needed[1].

The intensification of legume symbiotic nitrogen fixation in modern farming systems can not only be achieved by directed cultivation of legumes species and complementary strains of nodule bacteria, which are used for pre-seed nitrogenization taking into account the specific soil, climatic and agro-technical conditions, but also by creating mixed preparations on their basis[2].

Preparations based on nodule bacteria and natural, synthetic physiologically active substances are successfully introduced into the production[3]. The evidence about the more efficient impact of the microorganism association on the plant than other separate strains facilitated the emergence of a new generation of biological products based on micro-organisms, these are phosphate-mobilizing bacteria and *Bradyrhizobium*

[1] Агроэкологическая роль азотфиксирующих микроорганизмов в аллелопатии высших растений / В.Ф. Патыка и др.; под ред. В.Ф. Патыки. К.: Основа, 2004. 320 с.

[2] Коць С.Я., Патика В.П. Біологічна фіксація азоту та її значення в азотному живленні рослин. *Фізіологія рослин: проблеми та перспективи розвитку: в 2 т.* / гол. ред. В.В. Моргун. К.: Логос, 2009. Т. 1. С. 344–386.

Патыка В.Ф., Толкачев Н.З., Бутвина О.Ю. Основные направления оптимизации симбиотической азотфиксации в современном земледелии Украины. *Физиология и биохимия культурных растений*. 2005. Т. 37. № 5. С. 384–393.

[3] Векірчик К.М., Конончук О.Б. Стан і перспективи досліджень впливу обробки насіння БАР та інокуляції ризобіями на азотфіксацію, ріст, розвиток і продуктивність квасолі звичайної і сої культурної в умовах Тернопільської області. *Фізіологія рослин в Україні на межі тисячоліть: у 2 т.* / гол. ред. В.В. Моргун. К., 2001. Т. 1. С. 231–236.

Волкогон В.В. та ін. Нові біологічні препарати комплексної дії на основі активних штамів азотфіксувальних бактерій та фізіологічно активних речовин. *Фізіологія рослин: проблеми та перспективи розвитку: в 2 т.* / гол. ред. В.В. Моргун. К.: Логос, 2009. Т. 1. С. 393–403.

japonicum, which will allow to form more powerful symbiotic apparatus in soybean than with the help of monoinoculation[4], symbiotic and associative diazotrophs, rhizobia and Vesicular-Arbuscular Mycorrhizal Fungi[5], (*Cyanobacteriae*) blue-green algae and nodule bacteria, that have a positive multifunctional impact on plants etc.[6]

Considering the problem of creation of new multifunctional microbial preparations with nitrogen-fixing, growth-stimulating and bioprotective properties to increase crop yields and referring to the issue of obtaining high quality products and improving the environment, the use of nitrogen-fixing simbiotrof with EM-preparations (from EM – effective microorganisms). The developer of EM-technology is a Japanese scientist Teruo Higa, who united 86 species of anaerobic and aerobic microorganisms in preparation. His dominant group contains photosynthetic and lactic bacteria, actinomycetes, yeast and other enzyme-forming fungi etc. Each of these components performs its inherent function and interacts with other components and is useful for both plants and soil in its own way[7]. Ten years later, R. Buldagaeva and A. Nechesov, the scientists from Irkutsk (Russia), obtained a similar to EM-preparation – "Baikal EM-1U". The first experiments with the newly made biological product were conducted under the supervision of P. Shablin and showed its high efficiency. Russian preparation, compared to Japanese product, is marked by the predominance of lactic acid bacteria over photosynthetic, not vice versa. Therefore, the Japanese preparation had slightly better effect on plant growth, but the Russian, as it was conceived by the authors, contributes to faster soil

[4] Леонова Н.О. та ін. Ефективність застосування нітрагіну і регуляторів росту рослин при вирощуванні сої. *Сільськогосподарська мікробіологія*. 2007. Вип. 5. С. 74–85.

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[6] Воробей Н.А., Пацко О.В., Коць С.Я., Паршикова Т.В. Фізіологічні особливості розвитку люцерни за інокуляції змішаними культурами азотфіксувальних мікроорганізмів. *Физиология и биохимия культурных растений*. 2009. Т. 41. № 4. С. 344–352.

[7] Auerbach R. Organic agriculture a handbook. LINDROS Contact Information. URL: http://lindros.co.za/what-we-do/books/organic-agriculture-handbook/ (date of access: 18.03.2018).

Higa T., Parr James F. Beneficial and effective microorganisms for a sustainable agriculture and environment. Atami, Japan: International Nature Farming Research Center, 1994. 25 p. URL: http://www.em-la.com/archivos-de-usuario/base_datos/em_for_sustainable_agriculture_environment.pdf (date of access: 14.03.2018).

clearing from harmful substances and pathogenic microorganisms[8]. "Baikal EM-1U" is produced in Ukraine under the name of "Baikal EM-1U" Ltd. "EM Center, Ukraine" (Kharkiv) in the form of a ready solution with active microorganisms and a concentrate where live parts are at rest and require nutrient medium for activation[9].

The aim of our research work was to establish the expediency of two-component pre-treatment of *Glycine max* (L.) Merr., *Phaseolus vulgaris* L. and *Lupinus albus* L. of *Bradyrhizobium* and *Rhizobium* strains and the use of "Baikal EM-1U" in soil and climatic conditions of Ukraine Forest-Steppe.

1. Research results of bean

The research shows that EM enhances the diversity of beneficial soil microorganisms, and improves soil quality and the growth, yield and protection of crops[10].

Field experiments were conducted on black soil (chornozem) with low amount of humus and high loamy mechanical structure of agrobiolaboratory in Volodymyr Hnatiuk Ternopil National Pedagogical University. Contents of N (according to Cornfield) – 13,5 mg/100 g soil (low), P and K (according to Chirikov), respectively, 14,8 and 11,4 mg/100 g of soil (high), Mn – 68,5 mg/kg soil (middle), B – 1,21 mg/kg, Cu – 3,64 mg/kg soil (high), exchange acidity pH 6,7 (neutral).

"Baikal EM-1U" (TU 24.1-22700554-001-2003) was used in 1 to 100 for the humidification of the surface or 1 to 1000 for 1-hour soaking of seeds[11].

[8] Шаблин П.А. Эффективные микроорганизмы – надежда планеты. М.; Улан-Удэ: ООО «ЭМ-центр»; ПО «ЭМ-кооперация», 2000. 34 с.

[9] Пакулов К.Н. и др. ЭМ-технология в растениеводстве. Х.: АО «Центр испытательной техники», 2002. 20 с.

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[10] Higa T., Parr James F. Beneficial and effective microorganisms for a sustainable agriculture and environment. Atami, Japan: International Nature Farming Research Center, 1994. 25 p. URL: http://www.em-la.com/archivos-de-usuario/base_datos/em_for_sustainable_agriculture_environment.pdf (date of access: 14.03.2018).

Okubo H. Kyusei Nature Farming: Historical Perspective, Present Status and Prospects for Future Development with EM Technology / International Nature Farming Research Center. URL: http://www.infrc.or.jp/english/KNF_Data_Base_Web/PDF%20KNF%20Conf%20Data/C3-6-092.pdf (date of access: 15.03.2018).

An increase in the growth processes, after the simultaneous application of "Baikal EM-1U" and rhizobia, was possible mainly due to the increase in total area and dry mass of leaves on plants, dry weight of roots and stems. Biopreparation "Baikal EM-1U" significantly increased not only the same parameters but also the net productivity of photosynthesis, by 39,8% to control. Inoculation of *Rhizobium phaseoli* revealed significant stimulus only on the formation of leaf surface area and on the mass of root system (Table 1).

Table 1

The influence of inoculation and fertilizer "Baikal EM-1U" on the growth processes of *Phaseolus vulgaris* Nadiya, % to control

Indicator	"Baikal EM-1U"	St. 700	"Baikal EM-1U"+ st. 700
density of shoots	106,2	98,4	109,4
density of plants during the harvest	105,4	107,3	100,0
plant height[4]	109,5	102,9	109,1
leaves area[3]	124,9	135,4	120,6
stem base diameter[3]	102,5	98,5	102,0
the weight of dry leaves from one plant[3]	110,3	94,5	115,6
the weight of dry stem without the leaves[3]	124,3	103,8	112,1
the weight of dry root[3]	133,9	111,7	135,1
net product. of photosynthesis[3]	139,8	88,5	101,2
average	117,4	104,6	111,7

Note: [2] – phase of budding and blooming; [3] – phase of budding, flowering and green beans; [4] – phase of the fourth leaf, bud, blossom and full ripening

The study of leaf pigments showed that "Baikal EM-1U" had the highest stimulating effect during the phase of budding – the rise of chlorophyll *a* and main carotenoids was, respectively, 19,5 and 23,3%, "Baykal EM-1U" in combination with rhizobia – 15,6 and 13,2% and monoinoculation with rhizobia – 7,5 and 13,7% to control. In the phase of budding, the analyzed factors didn't show any significant effect on chlorophyll *b* (Table 2).

[11] Спосіб обробки насіння сої культурної: пат. 106442 Україна: МПК A01C 1/06 (2006.01) C12N 1/20 (2006.01). № u2015 10665; заявл. 02.11.2015; опубл. 25.04.2016, Бюл. № 8.

Table 2

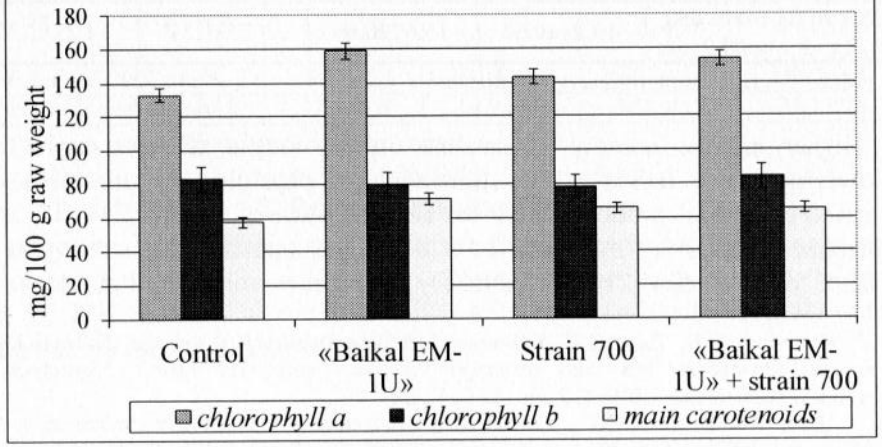
The amount of leaf pigments in *Phaseolus vulgaris* Nadiya under the influence of "Baikal EM-1U" and inoculation in the bud stage, mg/100 g raw weight

Indicator	Control	"Baikal EM-1U"	St. 700	"Baikal EM-1U"+ st. 700
chlorophyll *a*	133,0±8,0	159,0±4,0*	143,0±3,5	153,8±6,7
chlorophyll *b*	83,3±3,2	79,9±3,4	78,3±6,2	84,0±4,7
main carotenoids	57,6±2,2	71,0±3,6*	65,5±0,9*	65,2±6,5

*Note: * – significant difference from control*

Phase of flowering is distinguished by strengthening the combined pre-treatment of bean seeds with "Baikal EM-1U" and rhizobia and reducing the effect of monocultivation with "Baikal EM-1U". Thus, the combined effect of investigated factors led to significant increase in chlorophyll *a* 28,5%, chlorophyll *b* – 47,4% and the main carotenoids – 17,9% of the control, inoculation with rhizobia – 16,4, 10,0, 3,3% and with "Baikal EM-1U" – only chlorophyll *a* by 5,3% to the control (Figure 1).

Figure 1. The amount of leaf pigments in *Phaseolus vulgaris* Nadiya under the influence of «Baikal EM-1U» and inoculation in the flowering stage



The most remarkable stimulating effect on the formation and functioning of the legume-rhizobium symbiosis between *Phaseolus vulgaris* Nadiya and *Rhizobium phaseoli* bacteria[12] was found in the flowering stage (Table 3).

The functional activity of tubercles, which was evaluated according to the general nitrogenase activity (GNA)[13], showed the highest activity after the combined treatment with the microbes – the growth was 87,0% to control, monoinoculation with rhizobia – 58,7% and there was no change after using "Baikal EM-1U". At the same time, specific nitrogenase activity (SNA)[14] of the tubercles significantly increased by 34,1%, only in the variant when *Rhizobium phaseoli* were solely applied (Table 4).

Table 3

The legume-rhizobium symbiosis of *Phaseolus vulgaris* Nadiya after using "Baikal-EM-1U" fertilizer and inoculation at flowering stage

Indicator	Control	"Baikal EM-1U"	St. 700	"Baikal EM-1U"+ st. 700
number of tubercles, pieces/plant	29,8±2,3	50,3±4,9*	61,0±4,2*	82,8±7,1*
dry weight of tubercles, mg/plant	42,8±1,93	59,7±4,93*	67,3±0,99*	113,8±3,81*
dry weight of one tubercle, mg	1,47±0,10	1,15±0,11*	1,16±0,08*	1,45±0,13
GNA of tubercles mkg N_2/plant/year	0,92±0,03	0,92±0,09	1,46±0,11*	1,72±0,09*
SNA of tubercles mkg N_2/1g. dry weight/year	16,21±0,64	15,67±0,86	21,73±1,24*	14,23±1,25

*Note: * – significant difference from control*

Higher enzyme activity of catalase in the leaves of legumes in the flowering phase indicates the stimulation of physiological processes of studied biological product – after the combined use of "Baikal EM-1U" and rhizobia by 27,1%, "Baikal EM-1U" – 26,0% and rhizobia strain 700 – 18,3% to the control (216,8±15,2 ml O_2 per 1 g raw weight for 3 min.).

[12] Конончук О.Б., Пида С.В., Григорюк І.П. Вплив композиції добрив «Байкалу ЕМ-1У» та «Ризобофіт» на сою культурну (*Glycine max* (L.) Merr.). *Біоресурси і природокористування*. 2010. Т. 2. № 1/2. С. 12–21.

[13] Hardy R.W.F., Burns R.C., Holsten R.D. Application of the acerylene-ethylene assay for measurement of nitrogen fixation. *Soil Biol. Biochem*. 1973. Vol. 5. № 1. P. 41–83.

[14] Умаров М.М. Ацетиленовый метод изучения азотфиксации в почвенно-микробиологических исследованиях. *Почвоведение*. 1976. № 11. С. 92–95.

The main criterion of the effective influence of a certain factor on the plant is its impact on productivity. Thus, the study of biological value of bean grain yield showed that the highest growth at 3,6 cwt/ha was after the combined use of rhizobia and "Baikal EM-1U". Monocultivation with "Baikal EM-1U" and rhizobia turned out to be less effective – growth, respectively, by 1,4 and 1,2 cwt/ha (Table 4).

The analysis of elements of productivity illustrates that the highest indicated productivity growth of beans after combined application of biological products was mainly due to the high growth of aboveground crop mass – by 29,1%, the number of beans per one plant – 21,9% and the number of seeds per one plant – 33,2%.

Table 4

Key elements of productivity of *Phaseolus vulgaris* Nadiya after applying "Baikal EM-1U" fertilizer and inoculation

Indicator	Control	"Baikal EM-1U"	St. 700	"Baikal EM-1U"+ st. 700
density of plants, cwt/ha	305,6±14,0	322,2±14,3	327,8±10,6	305,6±14,0
biological yield without the leaves, cwt/ha	26,1±2,0	30,2±2,4	32,2±3,1	33,7±2,5
number of beans per one plant, unit	6,4±0,3	6,9±0,4	7,4±0,4	7,8±0,5
number of seeds per 1 plant, unit	26,8±1,6	31,0±1,7	32,9±2,0	35,7±2,4
number of seeds in a bean, pcs	4,1±0,09	4,5±0,10	4,4±0,10	4,5±0,10
weight of 1000 seeds, g	186,2±6,1	191,6±4,0	197,2±4,1	186,2±6,0
biological crop, cwt/ha	17,5±0,6	18,9±1,0	18,7±0,7	21,1±1,5

*Note: * – significant difference from control*

2. Research results of soybean

Effective microorganisms is a mixed culture of beneficial microorganisms that can be applied as an inoculant to increase the microbial diversity of soils. This in turn, can improve soil quality and health, which enhances the growth, yield, and quality of crops[15].

[15] New Biosphere Agriculture. Technology and Product Information. Queensland, Australia: Pascas Foundation (Aust) Ltd, 2002. 24 p. URL: https://www.pascasworldcare.com/index.php/new-biosphere-agriculture.html (date of access: 14.03.2018).

There has been established greater efficiency of the binary fertilizer "Baikal EM-1U" and inoculation application for sowing of the Annushka soybean variety to the growth processes (Table 5).

The study of leaf pigment amount in different phases of growth of soybean plants showed the most significant impact of the factors during the phase of green beans (Figure 2).

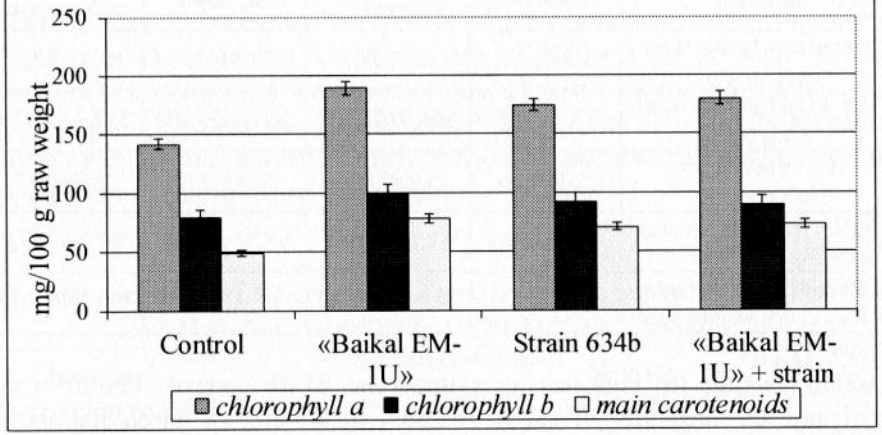
Table 5

Growth processes of *Glycine max* Annushka after the use of "Baikal EM-1U" and inoculation, % to control

Indicator	"Baikal EM-1U"	St. 634b	"Baikal EM-1U"+ st. 634b
germination	118,7	110,3	123,5
field similarity	118,0	109,8	123,0
height[5]	110,0	109,9	111,8
stem base diameter[3]	102,0	106,1	110,7
weight of dry stems without leaves[3]	118,8	120,7	130,5
dry root weight[3]	117,9	114,4	125,5
dry leaves weight[3]	115,0	124,4	121,0
leaves area[3]	105,9	123,5	125,1
average	113,3	114,9	121,4

Note: [3] – phase of budding, flowering and green beans; [5] – phase of fourth leaf, budding, flowering, green bean and full ripening

Figure 2. The amount of leaf pigments in *Glycine max* Annushka after the use of «Baikal EM-1U» and inoculation in the green bean stage



Under the influence of bioproduct "Baikal EM-1U" an increase in chlorophyll *a* was 33,5%, chlorophyll *b* – 24,5%, carotenoids – 56,9% to control. Rhizobia strain 634b increased the amount of these pigments, respectively, by 23,0, 15,9 and 43,3%, and combined application of rhizobia and biological fertilizer – by 27,1, 12,5 and 46,5% to control. In all tested variants significant increase in chlorophyll a and carotenoids was observed, which is due to the protective effect and extending of the operation of the photosynthetic apparatus of studied factors[16].

The study of legume-rhizobium symbiosis of *Glycine max* Annushka showed that fertilizer «Baikal EM-1U» and strain of *Bradyrhizobium japonicum* 634b significantly stimulated the formation of tubercles and their activity (Table 6), as well as other regulators of plant growth[17].

[16] Векірчик К.М., Конончук О.Б. Стан і перспективи досліджень впливу обробки насіння БАР та інокуляції ризобіями на азотфіксацію, ріст, розвиток і продуктивність квасолі звичайної і сої культурної в умовах Тернопільської області. *Фізіологія рослин в Україні на межі тисячоліть: у 2 т.* / гол. ред. В.В. Моргун. К., 2001. Т. 1. С. 231–236.

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[17] Леонова Н.О. та ін. Ефективність застосування нітрагіну і регуляторів росту рослин при вирощуванні сої. *Сільськогосподарська мікробіологія*. 2007. Вип. 5. С. 74–85.

Table 6

Legume-rhizobium symbiosis of *Glycine max* Annushka after the use of "Baikal EM-1U" and inoculation in the green bean stage

Indicator	Control	"Baikal EM-1U"	St. 634b	"Baikal EM-1U"+ st. 634b
number of tubercles, pieces/plant	64,8±5,1	57,7±5,7	79,6±4,2*	107,3±5,2*
dry weight of tubercles, mg/plant	338,4±16,56	525,9±25,55*	606,3±26,18*	593,3±15,72*
dry weight of one tubercle, mg	5,4±0,36	9,7±0,85*	7,6±0,31*	5,7±0,47
GNA of tubercles mkg N_2/plant/year	42,76±3,84	61,15±5,56*	68,23±4,56*	80,20±7,92*
SNA of tubercles mkg N_2/1g. dry weight/year	128,05±9,82	143,92±12,44	132,02±12,73	135,84±10,36

Thus, despite the high number of tubercles in the control version, which indicates the high titer of native rhizobia in the soil of agrobiolaboratory field, there was found a growing number of tubercles by 65,6% of control after the combined application of biofertilizer and rhizobia and by 22,8% – after monoinoculation during the phase of the green bean. Weight of dry tubercles per one plant was growing by 75,3% of control after the combined treatment, and by 79,2% – after monoinoculation and by 55,4% – only after using "Baykal EM-1U". It should be taken into account that in the latest version the growth of total amount of rhizobia took place due to an increase of 79,6% to the control value of tubercles, which was assessed by their dry weight, not by their quantity and weight, as in the other two variants.

The legume-rhizobium symbiosis which appeared after the application of "Baikal EM-1U" was characterized by higher activity than that formed by the same indigenous bacteria in the control plants. During the phase of green beans general nitrogenase activity increased by 43,0% and the specific nitrogenase activity – by 12,4% of control. The tubercle that were formed mainly by the industrial strain of *Bradyrhizobium japonicum* 634b had higher by 59,6% GNA and SNA – 3,1% to control. Combined presowing treatment with "Baikal EM-1U" and rhizobia formed the symbiosis, which was marked with the highest GNA – by 87,6% and SNA – by 6,1% to control.

The carried-out research confirmed the interdependence between the forming of soybean yield of *Glycine max* Annushka and the use of bio-fertilizer "Baikal EM-1U" and its combined use with the strain 634b (Table 7).

Thus, presowing treatment of soybean seeds with biological fertilizer "Baikal EM-1U" promoted the growth of grain productivity by 5,9 cwt/ha compared with the control. Combined use of "Baikal EM-1U" with rhizobia of production strain 634b increased the yield by 10,0 cwt/ha, which indicates the synergistic effect of the studied factors. Bacteria of strain 634b increased productivity by 0,5 cwt/ha, which shows the absence of the benefits of applied strain over local indigenous *Bradyrhizobium*.

Table 7

The key elements of *Glycine max* Annushka crop productivity after the use of bio-fertilizer "Baikal EM-1U" and inoculation

Indicator	Control	"Baikal EM-1U"	St. 634b	"Baikal EM-1U"+ st. 634b
density of plants, cwt/ha	305,6±10,6	338,9±10,6*	344,4±6,4*	372,2±14,0*
biological yield without the leaves, cwt/ha	48,8±2,6	58,±2,8*	49,5±3,7	63,9±6,0*
number of beans per one plant, unit	33,8±2,0	35,9±2,5	29,1±2,0*	35,2±2,7
number of seeds per 1 plant, unit	67,5±4,5	72,3±5,2	57,5±3,8*	71,1±5,8
number of seeds in a bean, pcs	2,00±0,07	2,02±0,06	2,00±0,05	2,03±0,06
weight of 1000 seeds, g	138,0±2,8	140,8±4,5	134,5±2,2	142,8±4,5
biological crop, cwt/ha	27,4±1,6	33,3±2,0	27,9±2,0	37,4±2,9*

*Note: * – significant difference from control*

The analysis of elements of productivity showed that the increase in grain productivity of *Glycine max* Annushka with combined application of "Baikal EM-1U" and nodule bacteria was possible primarily due to steady growth of stalk density of 21,8% and increase of aboveground plant weight of 30,9% to control, which are important factors of complete legumes crop productivity. "Baikal EM-1U" increased the stalk density by 10,9% as well

and aboveground mass by 19,5% compared with control plants. Plant inoculated solely by rhizobia formed 12,7% thicker crops in comparison with control.

The obtained findings indicate that the combined use of microbial fertilizers increased the indicators of both stalk density and crop yield of aboveground plant mass more efficiently than the use of "Baikal EM-1U" solely.

3. Research results of lupine white

Investigation of growth processes of *Lupinus albus* (variety Makarivskyi) during vegetation after mono- and combined pre-seed treatment by *Bradyrhizobium* sp. (*Lupinus*) strain 367a and the biopreparation "Baikal EM-1U" showed a stimulating effect on the growth of overground vegetative organs of plants. The height of the plant stem compared with the control in the phase of budding, flowering and green beans was higher by 6,5%, 14,6% and 33,3%, respectively, for the combined treatment of *Bradyrhizobium* sp. (*Lupinus*) strain 367a and biotype "Baikal EM-1U"[18]. A similar pattern was revealed considering the number of leaves per plant. *Bradyrhizobium* sp. (*Lupinus*) and the biopreparatum contributed to the branching of the stem of the plants. The number of lateral shoots of the plant experimental variants significantly differed from the control. Mono-application of "Baikal EM-1U" and *Bradyrhizobium* sp. (*Lupinus*) strain 367a in the phase of budding had the most significant increase in the mass of the stem with leaves (41,3 and 45,8%), while in the flowering phase the combination of "Baikal EM-1U" and *Bradyrhizobium* sp. (*Lupinus*) strain 367a had the best effect (54,1%). The mass of raw leaves per plant in the phase of budding was greater by 38,6% comparing to control after application of *Bradyrhizobium* sp. (*Lupinus*) strain 367a alone, while in the flowering phase a 40,7% increase for the combined influence of "Baikal EM-1U" and *Bradyrhizobium* sp. (*Lupinus*) strain 367a was observed. The mass of the dry stem in the phase of budding was higher (by 27,5%) under the influence of "Baikal EM-1U", and in the flowering phase under the action of combination of *Bradyrhizobium* sp. (*Lupinus*) strain 367a and "Baikal EM-1U" (by 46,6%).

Investigation of the amount of plastid pigments showed that in the phase of budding after monoprocessing of seeds with biopreparation "Baikal EM-1U" no significant difference in the content of chlorophylls *a* and *b* was detected in the leaves of *Lupinus albus* in comparison with control.

Inoculation of seed with *Bradyrhizobium* sp. (*Lupinus*) and its combined use with the "Baikal EM-1U" contributed to chlorophyll increase by 12,8 and 15,8%, respectively, and 6,2 and 14,2%, respectively (Table 8). The flowering phase revealed a similar pattern in the accumulation of pigments in the leaves of *Lupinus albus*.

Table 8

The amount of leaf pigments in *Lupinus albus* Makarivskyi under the influence of "Baikal EM-1U" and inoculation in the bud stage, mg/100 g raw weight

Indicator	Control	"Baikal EM-1U"	Strain 367a	"Baikal EM-1U"+ st. 367a
chlorophyll *a*	241,4±8,4	266,0±4,9	272,4±8,0 *	279,6±7,7*
chlorophyll *b*	153,5±6,2	163,5±4,7	163,0±5,2	175,3±8,7*
main carotenoids	71,1±4,3	83,5±2,6*	89,5±4,9*	85,2±5,4

*Note: * – significant difference from control*

Important indicators characterizing the effectiveness of the symbiotic system are the mass of root nodules and their nitrogen activity. It has been established that the use of *Bradyrhizobium* sp. (*Lupinus*) standard strain 367a and its combined application with the biopreparation "Baikal EM-1U" caused formation of the nodules mainly on the main tap root of *Lupinus albus* and all nodules had pink color. Interestingly, in the soil of experimental sites there were local races of nitogen fixing bacteria that spontaneously inoculated the roots of the plants of the control and experimental "Baikal EM-1U" variants. Biopreparation "Baikal EM-1U" intensified the plant's ability to be infiltrated by bacteria and promoted the growth of nodules on the roots of white lupine, both during spontaneous inoculation and in combination with the introduced strain. *Bradyrhizobium* sp. (*Lupinus*) strain 367a in our soil-climatic conditions was characterized

[18] Бабачук Н.Б., Костриба І.І., Дудчук І.В., Пида С.В. Ростові процеси люпину білого за застосування композицій ризобофіту та «Байкалу ЕМ-1У». *Екологічні проблеми сільськогосподарського виробництва: матеріали V всеукр. наук.-практич. конф. молодих учених* (Яремче, 21–24 червня 2011 р.). К.: Інститут агроекології і природокористування НААНУ, 2011. С. 170–172.

by high virulence and contributed to the formation of active nodules in the form of large and heavy structures. The highest activity of the symbiotic system "*Lupinus albus – Bradyrhizobium* sp. (*Lupinus*)" were shown in the phase of budding, which is consistent with previous studies[19].

It was established that in the budding phase, pre-sowing inoculation of seeds increased the weight of nodules on plant roots by 36,6%, treatment with biopreparation – by 18,0% and their combined application – by 66,6% (Table 9). A significant increase in total and specific nitrogenase activity was detected by monoinoculation and co-administration of *Bradyrhizobium* sp. (*Lupinus*) strain 367a and biopreparation "Baikal EM-1U".

Table 9

The legume-rhizobium symbiosis of *Lupinus albus* Makarivskyi after use of "Baikal-EM-1U" bioprepation and inoculation at budding stage

Indicator	Control	"Baikal EM-1U"	St. 367a	"Baikal EM-1U"+ st. 367a
dry weight of nodules, mg/plant	56,6±3,3	66,8±3,9	77,3±1,9*	94,3±1,1*
GNA of nodules mkg N_2/plant/year	0,89±0,07	1,02±0,08	1,96±0,11*	2,21±0,012*
SNA of nodules mkg N_2/1g. dry weight/year	15,72±0,44	15,27±0,86	25,36±1,24*	23,44±1,25*

*Note: * – significant difference from control*

Seed productivity is an important criterion for evaluating the formation and functioning of symbiotic legume crops systems[20]. The highest increase in *Lupinus albus* seed yield at 6,8 cwt/ha was observed for combined use (Table 10). Monoinoculation of seeds with *Bradyrhizobium* sp (*Lupinus*) strain 367a or seed treatment with "Baikal EM-1U" biopreparation was less effective (yield increase comparing to control at 18,6 and 10,1%, respectively). The increase in seed productivity is associated with an increase in the density of plants, the number of beans on plants of experimental variants, and a mass of 1 000 seeds.

[19] Пида С.В., Тригуба О.В., Кончук О.Б. Формування і функціонування симбіотичної системи *Lupinus albus* L. – *Bradyrhizobium* sp. (*Lupinus*) за використання ризобофіту і рістрегуляторів. *Наукові записки Тернопільського національного педагогічного університету імені Володимира Гнатюка. Серія «Біологія».* 2014. № 3(60). С. 156–161.

[20] Біологічний азот / В.П. Патика та ін.; за ред. В.П. Патики. К.: Світ, 2003. 424 с.

Table 10

The key elements of *Lupinus albus* Makarivskyi crop productivity after the use of bio-fertilizer "Baikal EM-1U" and inoculation

Indicator	Control	"Baikal EM-1U"	St. 634b	"Baikal EM-1U"+ st. 634b
density of plants, cwt/ha	303,8±9,8	329,9±9,4*	351,3±6,8*	378,6±13,4*
number of seeds per 1 plant, unit	12,5±0,3	13,5±0,9	20,1±0,6*	24,6±0,9*
number of seeds in a bean, pcs	4,1±0,08	4,3±0,07	4,8±0,05	4,9±0,07
weight of 1 000 seeds, g	265,6±6,4	279,8±5,7	283,7±7,2	304,4±8,5*
biological crop, cwt/ha	23,7±1,4	26,1±1,9	28,1±1,2*	30,5±2,1*

*Note: * – significant difference from control*

CONCLUSIONS

Agriculture of the Ukraine faces many problems. Among the various types of solutions offered, the role of Kyusei Nature Farming with EM-technologies has a very important role. This is not indicated as a forecast, but with a very successful experience in all parts of this world.

The leading role in the improvement of nitrogen nutrition of plants and increase of soil fertility should be given to the cultivation of leguminous crops, in particular, ordinary beans and soybean culture, which should be considered not only in terms of obtaining basic products, but also as nitrogen fixators of the atmosphere.

The intensification of legume symbiotic nitrogen fixation in modern farming systems can not only be achieved by directed cultivation of legumes species and complementary strains of nodule bacteria, which are used for pre-seed nitrogenization taking into account the specific soil, climatic and agro-technical conditions, but also by creating mixed preparations on their basis.

Thus, presowing treatment of *Phaseolus vulgaris* Nadiya, *Glycine max* Annushka and *Lupinus albus* Makarivskyi in soil and climatic conditions of Ukraine Forest-Steppe with EM-fertilizer "Baikal EM-1U" and complementary production strains of nodule bacteria *Rhizobium phaseoli* strain 700, *Bradyrhizobium japonicum* strain 634b and *Bradyrhizobium* sp (*Lupinus*) strain 367a showed that predominantly more effective, according

to the complex of physiological and biochemical parameters and the level of crop productivity, is the combined use of bioproducts than their sole use.

The results obtained during the research enable us to suggest a combined presowing treatment with microbiological fertilizer "Baikal EM-1U" and the inoculation of legume seeds as a perspective tendency of crop productivity growth.

SUMMARY

The article deals the problem of creation of new multifunctional microbial preparations with nitrogen-fixing, growth-stimulating and bioprotective properties to increase crop yields and referring to the issue of obtaining high quality products and improving the environment, the use of nitrogen-fixing simbiotrof with EM-preparations (from EM – effective microorganisms).

The results obtained during the research enable us to suggest a combined treatment of *Phaseolus vulgaris* Nadiya and *Glycine max* Annushka in soil and climatic conditions of Ukraine Forest-Steppe with EM-fertilizer "Baikal EM-1U" and the inoculation of nodule bacteria *Rhizobium phaseoli* strain 700 and *Bradyrhizobium japonicum* strain 634b of seeds as a perspective tendency of crop productivity growth (increased the yield by 3,6-10,0 cwt/ha) and nitrogen-fixing.

Presowing treatment of white lupine seeds (variety Makarivskyi) with fertilizer "Baikal EM-1U" in combination with nitrogen-fixing bacteria *Bradyrhizobium* sp (*Lupinus*) strain 367a contributed to the branching of the stem, the growth of leaves on the plant, increased chlorophyll a content, increased mass of nodules on the roots and their nitrogen activity.

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