Effectiveness of polyfunctional microbial preparations in growing *Pisum sativum* L. in the South of Russia

S V Didovich, O P Ptashnik and V S Pashtetskiy

Research Institute of Agriculture of Crimea, 150, Kievskaya str., Simferopol, 295493, Russia

E-mail: didovich_s@niishk.ru

Abstract. The possibility of obtaining high yields of pea (*Pisum sativum* L.) with bacterial pre-sowing treatment without the use of mineral fertilizers in the steppe zone of the Crimea was shown. Effectiveness of microbial preparations in unfavorable climatic conditions in 2015, 2017 and favorable conditions in 2016 in the steppe zone of the Crimea was determined. On average, for 2015-2017, the use of pre-sowing treatment with Cyanorhizobial consortium was provided a significant increase in 1000 seeds weight by 18.92 g (8.7%) compared to the variant with a mineral fertilizer Amophos (Duncan’s test, p = 0.000053). Application of polyfunctional complex Rhizobofit + Phosfoenterin + Biopolicyd and Cyanorhizobial consortium contributed to a significant increase in seed yield by 0.14-0.16 t/ha (5.47-6.257%) compared to a mineral fertilizer Amophos (Duncan’s test, p = 0.000077; p = 0.000226). These preparations are recommended for growing pea in the steppe zone of the Crimea.

1. Introduction

Pea (*Pisum sativum* L.) is the most important source of plant protein for animal feeding and human nutrition. According to Russian Statistics, pea occupy 53% (1.5 million ha) of the total area of legumes in Russia [1]. The pea forms a unique symbiotic relationship with nodule bacteria of the species *Rhizobium leguminosarum* and can assimilate up to 70 kg/ha of nitrogen from the air annually, meeting its needs for this element by 50% and leaving up to 10-15% of the symbiotic nitrogen in the soil with crop residues for subsequent agricultural crops [2, 3]. This allows saving up to 20% of nitrogen fertilizers. Pre-sowing treatment of seeds with microbial preparations based on highly effective rhizobia strains should be used for the effective process of biological nitrogen fixation and increasing the productivity of pea. In addition, the remaining reserve of productivity can be obtained by optimizing the conditions for the functioning of the symbiosis by improving phosphorus nutrition, growth simulation, selection of varieties and agricultural technologies. In Russia, there is a long-term practice of using microbial preparations in the cultivation of legumes [4-6].

A collection of promising strains of nodule bacteria for almost all legumes grown in Russia and abroad has been created, maintained and is constantly updated in the Department of Agricultural Microbiology of the Federal State Budget Scientific Institution "Research Institute of Agriculture of Crimea". These strains are included in the Crimean Collection of Microorganisms of the Scientific and Technological Infrastructure of the Russian Federation [7]. Polyfunctional microbial preparations are developed on the basis of collection strains of the useful bacteria.
The purpose of this study was to assess the effectiveness of microbial preparations based on nitrogen-fixing nodule bacteria, phosphatmobilizing and plant growth-promoting bacteria in growing pea in the steppe zone of the Crimea.

2. Materials and methods
The research was carried out in 2015-2017 in the experimental plots in the steppe zone of the Crimea on southern Chernozem region, with the humus content of 2.2-2.3%. The thickness of the humus layer is 50 cm. The arable soil layer (0-20 cm) was characterized by high availability of exchangeable potassium (32-36 mg/100 g of soil), average availability of mobile phosphorus (4.6-6.0 mg/100 g of soil) and low availability of easily hydrolyzed nitrogen (3.0-4.0 mg/100 g of soil); the reaction of soil solution was pH 7.5. Agrochemical parameters of soils were determined using standard methods: humus according to Tyurin, mobile phosphorus (P2O5) and exchangeable potassium (K2O) according to Machigin. The easily hydrolyzed nitrogen was determined according to GOST 26213-91 [8].

Microbial preparations for the experiments were made in the Department of Agricultural Microbiology of Federal State Budget Scientific Institution "Research Institute of Agriculture of Crimea" (Simferopol). Rhizobofit (R) is a microbial preparation based on symbiotic nitrogen-fixing nodule bacteria of the species Rhizobium leguminosarum. Phosfoenterin (Ph) is a preparation based on phosphate-mobilizing strain Lelliottia nimpressuralis. Biopolycid (B) is a microbial preparation based on the antagonist of phytopathogens strain Paenibacillus polymyxa. Cyanobacterial consortium (CRC) is a microbial preparation based on a specific strain of rhizobia Rhizobium leguminosarum and phototrophic nitrogen-fixing strain of cyanobacteria Nostoc linekia with 12 different functions in associative to this cyanobacteria strains. All used strains are plant growth-promoting bacteria. The biopreparations were used for pre-sowing treatment of seeds pea in an amount of 1.5 % of the working solution by seeds weight. As a control, the variant with application of mineral fertilizer Amosphos in doses of N5P3O10 before sowing.

Agro-climatic conditions of steppe zone are characterized by unstable and insufficient moisture, with a mean annual rainfall of 340-418 millimeters and a mean annual air temperature of 9.8-10.4 °C [9]. Mid-season pea variety Pharaoh was studied in our experiments. The experiments are based on three replications, with a plot area of 25 square meters, were carried out by common methods [10].

During the years of the experiments, the weather conditions differed from favorable (2016) to unfavorable (2015, 2017).

The amount of active air temperature in the growing seasons 2015 was 980 °C, which was higher than the long-time average annual air temperature by 35°C. The amount of precipitation was 322.3 mm, which was higher than the long-time average annual value by 119.3%. However, there was 17.5 mm less precipitation at the time of sowing. Hydrothermal index was at the level of 3.28.

The amount of active air temperature in the growing season 2016 was 1177.9 °C, this was higher by 232.9 °C in comparison with the long-time average annual value for the similar period. The amount of precipitation was 2.7 times higher than the long-time average annual value and reached 433.4 mm (long-time average annual value – 160 mm). Hydrothermal index was at the level of 3.67. Climatic regime in 2016 in the phases of development of pea was close to the optimal parameters and corresponded to its biological requirements.

In 2017, the amount of active air temperature was 1016.5 °C, which was higher than the long-time average annual temperature by 71.5 °C, however the amount of precipitation was 40.9 mm (72.2%) less than the norm. Hydrothermal index was at the level of 1.04.

Statistical processing of the research results was carried out by the method of dispersion analysis using the Statistica 10 software.

3. Results and Discussion
The parameters of the 1000 seeds weight and seed yield were estimated. In our experiments, these parameters were influenced by the vegetation conditions in a particular year and the pre-sowing treatment of the seed with microbial preparations.
The most favorable climatic conditions of vegetation were in 2015 and 2016, which contributed to the formation of the highest 1000 seeds weight, which, on average was 221.3-232.5 g. The lowest 1000 seeds weight was formed in the droughty 2017 and was 195.0-210.0 g (Figure 1).

![Method of Least Squares, average (year*preparation)](image)

**Figure 1.** Influence of *Pisum sativum* L. seeds bacterization by microbial preparation on the 1000 seeds weight (g): N₃₀P₃₀ – mineral fertilizer Amophos in doses of N₃₀P₃₀, R – Rhizobofit, CRC – Cyano-rhizobial consortium, Ph – Phosfoenterin, B – Biopolicyd.

On average, for three years, according to conservative posteriori Duncan’s test, microbial treatment of seeds with Cyano-rhizobial consortium significantly differed compared to control and other variants of bacterization (Table 1). Seeds bacterization with CRC contributed to a significant increase in 1000 seeds weight by 18.92 g (8.7%) compared to the variant with mineral fertilizer Amophos (p = 0.000053); by 6.84-7.17 g (2.9-3.0%) than in the variants with Rhizobofit (p = 0.000121) and microbial complex Rhizobofit + Phosfoenterin + Biopolicyd (p = 0.000063).

The yield after the use of bacterization is presented in Figure 2.

**Table 1.** Approximate probabilities for a posteriori Duncan’s test to the 1000 seeds weight on average, for 2015-2017

| Experimental variants | N₃₀P₃₀ (216.25 g) | R (228.00 g) | CRC (235.17 g) | R+Ph+B (228.33 g) |
|------------------------|------------------|--------------|----------------|------------------|
| N₃₀P₃₀                 | –                | 0.000121     | 0.000053       | 0.000063         |
| R                      | 0.000121         | –            | 0.000226       | 0.842301         |
| CRC                    | 0.000053         | 0.000226     | –              | 0.000328         |
| R+Ph+B                 | 0.000063         | 0.842301     | 0.000328       | –                |

Notes: 1000 seeds weight is shown in parentheses (on average, for three years), significant pair comparisons are highlighted in red, N₃₀P₃₀ – mineral fertilizer Amophos in doses of N₃₀P₃₀, R – Rhizobofit, CRC – Cyano-rhizobial consortium, Ph – Phosfoenterin, B – Biopolicyd.
Method of Least Squares, average (year*preparation)
Wilks' Lambda = 0.10158, F(12, 70) = 12.469, p = 0.00000
SEM are indicated in vertical columns

2015 2016 2017

Seed yield, t/ha
N 30
P 30
R
CRC
R+Ph+B

Figure 2. Influence of Pisum sativum L. seeds bacterization by microbial preparation on the seed yield, t/ha: N 30P 30 – mineral fertilizer Amophos in doses of N 30P 30, R – Rhizobofit, CRC – Cyano-rhizobial consortium, Ph – Phosfoenterin, B – Biopolicyd.

Climatic conditions affected the yield of Pisum sativum L. Seed yield in control amounted to 2.07 t/ha (2015) and 2.13 t/ha (2017). Seed yield in the studied experimental variants with inoculation of microbial preparations was 2.26-2.32 (2015) and 2.12-2.26 (2017). The low seed yield of Pisum sativum L. in 2015 and 2017 was because of unfavorable conditions that have developed in the growing season, namely low humidity of soil, air and low rainfall.

Maximum seed yield reached 3.48 t/ha in the wetter year of 2016. Seeds treatment with Cyano-rhizobial consortium and complex Rhizobofit + Phosfoenterin + Biopolicyd contributed to increase in seed yield by 0.12 t/ha (3.44%) (p < 0.05) compared to the variant with a mineral fertilizer. On average, for three years, according to conservative posteriori Duncan’s test, microbial treatment of seeds with polyfunctional complex Rhizobofit + Phosfoenterin + Biopolicyd and Cyano-rhizobial consortium significantly increased seed yield by 0.14-0.16 t/ha (5.47-6.257%) compared with the a mineral fertilizer "Amophos" (p = 0.000077; p = 0.000226), as can be seen from Table 2.

Table 2. Approximate probabilities for a posteriori Duncan’s test to the seed yield on average, for 2015-2017

| Experimental variants | N 30P 30 (2.5625 t/ha) | R (2.5867 t/ha) | CRC (2.6950 t/ha) | R+Ph+B (2.7167 t/ha) |
|-----------------------|------------------------|----------------|-------------------|---------------------|
| N 30P 30              | –                      | 0.436797       | 0.000226          | 0.000077            |
| R                     | 0.436797               | –              | 0.001297          | 0.000271            |
| CRC                   | 0.000226               | 0.001297       | –                 | 0.485345            |
| R+Ph+B                | 0.000077               | 0.000271       | 0.485345          | –                   |

Notes: seed yield is shown in parentheses (on average, for three years), significant pair comparisons are highlighted in red, N 30P 30 – mineral fertilizer Amophos in doses of N 30P 30, R – Rhizobofit, CRC – Cyano-rhizobial consortium, Ph – Phosfoenterin, B – Biopolicyd.

The study of the effectiveness of microbial preparations when growing pea has shown that we have succeeded in increasing weight of 1000 seeds and seed yield without the use of mineral fertilizers in the steppe zone of the Crimea.
4. Conclusion
Thus, the effectiveness of microbial preparations was high in unfavorable climatic conditions of 2015 and 2017 as well as in favorable conditions of 2016 when growing pea in the steppe zone of the Crimea.

On average, for three years, seeds bacterization with Cyano-rhizobial consortium contributed to a significant increase in weight of 1000 seeds by 18.92 g (8.7%) compared to the variant with a mineral fertilizer Amophos (Duncan’s test, p = 0.000053). Microbial treatment of seeds with polyfunctional complex Rhizobofit + Phosfoenterin + Biopolicyd and Cyano-rhizobial consortium significantly increased seed yield by 0.14-0.16 t/ha (5.47-6.257%) compared to with the mineral fertilizer Amophos (Duncan’s test, p = 0.000077; p = 0.000226). These preparations are recommended for growing Pisum sativum L. in the steppe zone of the Crimea.

References
[1] Bulletins on the state of agriculture (electronic versions) Catalog of publications of the Federal State Statistics Service, Available at: http://old.gks.ru/wps/wcm/connect/rosstat_main/rosstat/ru/statistics/publications/catalog/doc_1265196018516
[2] Babich A O, Petrichenko V F and Adamen F F 1996 The problem of photosynthesis and biological fixation of nitrogen by legumes Bulletin of agricultural science 2(514) 34-39
[3] Kokorina A L and Kozhemyakov A P 2010 Bean-Rhizobial Symbiosis and Application of Microbiological Preparations of Complex Action – An Important Reserve for Increasing Productivity of Arable Land (Saint-Petersburg)
[4] Zavalin A A 2005 Biopreparations, Fertilizers and Yield (Moscow)
[5] Tikhonovich I A, Borisov A Yu, Vasilchikov A G, Zhukov V A, Kozhemyakov A P, Naumkina T S, Chebotar V K, Shtark O Yu and Yakhno V V 2012 Specificity of microbiological preparations for legumes and features of their production Legumes and cereals 3 11-17
[6] Turina E L, Didovich S V and Kulininich R A 2015 Application of poly functional biologics in the cultivation of legumes Agriculture 2 31-33
[7] 2019 Collection of Microorganisms of Federal State Budget Scientific Institution "Research Institute of Agriculture of Crimea"USI: CCM FSBSI "RIAC", Sci. and Technol. Infrastruct. of the Russ. Feder. Centers for Collect. Use of Sci. Equipm. and Unique Sci. Installations, available at: http://ckp-rf.ru/usu/507484/
[8] 1992 GOST 26213-91 Soils. Methods for determination of organic matter (Moscow) Available at: http://docs.cntd.ru/document/1200023481
[9] Polovitsky I Ya and Gusev P G 1987 Soils of Crimea and increase of their fertility: Reference edition (Simferopol: Tavria)
[10] Dospekhov B A 2011 Methods of Field Research (Moscow)