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# EFFECTS OF COMBINED TREATMENT OF SEEDS OF TWO PEA VARIETIES WITH BACTERIAL INOCULANTS

## SUMMARY

The series of the field experiments on pea inoculation with three kinds of bacteria were conducted in Dnipropetrovsk province situated in the northern part of steppe zone of Ukraine. The main objective of our research was to study effects of combined seed treatment of two pea cultivars with bacterial inoculants.

The largest leaf surface area of pea cultivars was formed in the stage of flowering in the combine inoculation with bacterial strains *Rhizobium leguminosarum, Bacillus megaterium and Paenibacillus polymyxa* (PP). The three-component mixture provided nitrogen fixation with rhizobium (R) and phosphorus soluble mobilization (PSB) and has led to a significant increase both the total number of nodules (by 1.4-2.0 times) and their active forms (by 1.3-2.0 times) on the pea root system. The treatment of seeds of two pea varieties with bacterial inoculants made it possible to increase the yield of pea grain up to 13%. The effect of pea seeds bacterial inoculation against the $N_{20}P_{40}$ background was less effective.

The highest effect was recorded after inoculation of pea seeds with *Rhizobium leguminosarum*. The use of a double (PSB + PP) and triple mixture (PSB + PP + R) of bacterial inoculants provided the second place to an increase in the nitrogen content in the foliar mass of plants in both treatments (without and with mineral fertilizers). The amounts of phosphorus and potassium uptake in the foliar mass were higher in the PSB version (without fertilizers), and in the treatment with the use of *P.Polymyxa* - with the use of $N_{20}P_{40}$ fertilizers.

**Keywords**: pea cultivars, bacterial inoculants, yield, NPK uptake

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## INTRODUCTION

It was established that initial growth and subsequent $N_2$ fixation of pea may be enhanced by the addition of low amounts of N fertilizer prior to planting (Mahon and Child 1979). The application of effective bio-fertilizers increases the grain yield of leguminous crops when pea varieties with the most remarkable ability for symbiosis are included in the crop rotation (Mekonnen and Assefa, 2019). The presence of a short growing season in peas guarantees the formation of a certain period of time for the accumulation of moisture in the soil in a sufficient amount for the normal development of the next crop rotation. It is known that field peas can fix atmospheric nitrogen ($N_2$) due to a symbiotic relationship with bacteria *Rhizobium leguminosarum* (Mutch and Young, 2004; Peix *et al.,* 2015*)*. The tolerance of rhizobial isolates to different physic-chemical conditions makes it possible to select inoculants of rhizobial strains with ecological competitiveness (Kumar *et al.*, 2015; Bourion *et al.,* 2018). Bacterial inoculation with Rhizobium species allow to improve the root architecture of legume crop through their potential of colonization. Peas take up a start portion of nitrogen from soil reserves. McKenzie *et al.* (2001) found, that application of N fertilizer increased pea yield in 33% of the trials by an average of 11%, when spring soil $NO_3$-N to 30 cm was less than 20 kg N $ha^{-1}$.

The regulation of acid and alkaline phosphatases is critical for plant survival in soils with low level of phosphorus (Duff *et al.,* 1991). Dual roots colonization with arbuscular mycorrhizal fungi (AM) or phosphate-solubilizing bacteria (PSB) play key role to provide dual effect after seeds inoculation with *Rhizobium leguminosarum (*Geneva *et al*., 2011; Wang *et al*., 2017*)*. Synergistic effect of dual colonization of roots with AM fungi and *Rhizobium* on growth, nutrient uptake and nitrogen fixation in and pea was reported (Xavier and Germida, 2003). Mycorrhizal fungi hyphae secrete acid and alkaline phosphatases into the rhizosphere (Duff *et al.,* 1991). A numerous phosphate-solubilizing bacteria (PSB) also have the ability to dissolve insoluble P into an available form with some organic acids and secretion of enzymes to promote plant growth (Fürnkranz *et al.,* 2009; Seo and Park, 2009). Mainly, identified PSB belong to the *Pseudomonas, Bacillus, Mycobacterium, and Enterobacter* genera (Hanif et al., 2015). Last two decades the bacterial strains of *Pseudomonas spp.* attract more attention in the field of biofertilization because of their phosphate solubilization ability. The ability of *Pseudomonas spp.* and their consortium to solubilize insoluble inorganic and organic phosphate into absorbable form for Mung bean was shown (Kumar *et al.* 2017). The maximum seed and straw yields were recorded in combination *Pseudomonas* striate with 60 kg $P_2O_5$ $ha^{-1}$ in the field experiment with chickpea crop cultivation (Chauhan.and Raghav 2017). The joint influence of Rhizobium and phosphate solubilizing bacteria (PSB) were tested on Pea (*Pisum sativum* L.) with the incorporation of two levels of nitrogen (0 and 10 kg $ha^{-1}$) and two P sources at phosphate (Tyagi *et al*., 2003). Meantime the highest grain yield was fixed also in case of combined application of Rhizobium + PSB. So, the interaction effect between phosphorus and bio-fertilizers was established. The *Paenibacillus polymyxa* is a non pathogenic and endospore-forming Bacillus and can be efficiently used in supplying phosphorus (Lal and Tabacchioni, 2009). This strain can be efficiently used in rhizosphere competence (Mohd Din *et al.,* 2020).

The main objective was to study effects of two pea cultivars combined seed treatment with bacterial inoculants.

## MATERIAL AND METHODS

The series of field experiments with biostimulants were conducted in Verkhnedniprovsky district of Dnipropetrovsk province situated in the northern part of the steppe zone of Ukraine from 2006–2008 and 2015–2017 years (Lemishko 2020). This research was carried out in a six-field chain of grain-row crop rotation. After harvesting the predecessor of peas (winter wheat), disking was carried out to a depth of 10-12 cm, followed by shelf plowing to a depth of 20-22 cm. Early spring tillage consisted of leveling the soil, pre-sowing cultivation, and application of mineral fertilizers with a rate of $N_{20}P_{40}$ (ammonium nitrate, granular superphosphate). Sowing was carried out with a seeder with 15 cm row spacing at the optimal time when the soil was physically ripe. The object of research was the two pea cultivars Kharkivsky yantar and Kharkivsky etalon. The seed sowing rate was 1.5 million/ha.

The average annual rainfall is 455 mm. Their distribution during the year is does not match the information below uneven: 18% of the annual amount falls in winter, 23% in spring, 37% in summer, and 22% in autumn. Precipitation often comes in the form of showers and their efficiency is within 24-28%, and the rest of the moisture is spent on runoff and evaporation from the soil surface. The average annual air temperature is + 8.1ºC. The average temperature of the coldest month (January) is -5.6ºC, and the warmest (July) is + 20.5ºC. In some years, the maximum daytime air temperature in May is +21ºC, in August +27ºC, and sometimes even reaches +40ºC. The relative humidity from May to September ranges from 40 to 50%. In dry years, it sometimes decreases to 20-30%, and in wet years it increases to 60-70%. The content of humus in the top of black soil is 4.5%, total nitrogen - 0.26, phosphorus - 0.16, and potassium - 2.5%.

The scheme of the field experiment included control and five seeds bacterial inoculation separately, dual and triple: control; *Rhizobium leguminosarum* (R) at the dose of 200g/ton of seeds; *Bacillus megaterium* phosphorus solubilization bacteria (PSB) – 455ml/ton of seeds; *Paenibacillus polymyxa* (PP) – 600ml/ton of seeds; R+PSB; R+PP and R+PSB+PP. The control trial included seeds water treatment accepted in the field experiment. The area of the leaf surface was determined in the selected samples by the method of "cuts" in the following sequence. First, the weight of the leaves of all selected medium plants was determined. Then cutouts of these leaves, counting at least 100 pieces and weighing them were made. The area of leaves of one plant was calculated according to the formula (Nichiporovich, 1972):

$$S = W_l \cdot S_d / W_c \cdot n, \quad (1)$$

where S is the square of leaves of one plant, $cm^2$; $W_l$ - weight of leaves, g; $S_d$ - square of disks, $cm^2$; n - the number of selected plants that make up the average sample; Wc - is the weight of the cuttings, g.

The amount of active and inactive nodules per plant was calculated during vegetation season (Fischinger and Schulze, 2010). Nodules were separated according to their color (red to pink, active; greenish to brown, inactive). The nitrogen concentration in plant samples was estimated using Kjeldahl method, total P – by sulfuric acid digestion, and potassium – with flame photometry (Thomas *et al.,* 1967). N, $P_2O_5$, and $K_2O$ uptake with pea mass was accounted. Data received in field experiments accomplished were processed by statistical methods using the software package StatGraphics Plus5 with all tests of significance being made at a type 1 error rate of 5%.

## RESULTS AND DISCUSSION

A clear dependence of the formation of the leaf surface area of two pea cultivars on the influence of various bacterial fertilizers was observed on average over three years of field experiment (Table 1).

Table 1. The leaf surface area of pea cultivars depending on inoculation during the interval of vegetative growth, (thousand $m^2$/ha)

| Cultivars (factor A) | Bacterial fertilizer (factor B) | Growth stages | | | |
|---|---|---|---|---|---|
| | | 3rd leaf | Budding | Flowering | Grain ripeness |
| Kharkivsky yantar | Control | 1.0 | 26.8 | 41.7 | 9.4 |
| | PSB | 1.0 | 27.3 | 42.3 | 10.7 |
| | PSB+PP | 1.1 | 29.1 | 44.4 | 11.3 |
| | PSB+Rhisobium, (R) | 1.2 | 29.7 | 47.3 | 11.4 |
| | PSB+PP+R | 1.3 | 30.3 | 48.8 | 12.5 |
| Kharkivsky etalon | Control | 0.82 | 23.6 | 39.1 | 18.1 |
| | PSB | 0.98 | 28.6 | 43.1 | 10.0 |
| | PSB+PP | 1.0 | 29.3 | 42.4 | 10.9 |
| | PSB+Rhisobium, (R) | 1.1 | 29.7 | 46.7 | 11.2 |
| | PSB+PP+R | 1.2 | 29.4 | 47.9 | 11.8 |
| $LSD_{0,95,}$ thous $m^2$/ha | for factor A | 0.20 | 0.25 | 1.50 | 0.9 |
| | for factor B | 0.15 | 0.85 | 1.60 | 1.10 |
| | for AB interaction | 0.30 | 1.01 | 3.20 | 2.0 |

The largest leaf surface area of the pea plants was established at the stage of total flowering and varied within 38.7-52.6 thousand $m^2$/ha. The area of the leaf surface steadily increased starting from the seedling to the full flowering stages. Meanwhile, this tendency decreased significantly (almost 3-4 times) after the full grain maturity stage. The testing pea cultivars formed an unequal area of the leaf surface. The leaf surface area of the Kharkivsky yantar was 43.7 thousand $m^2$/ha in the flowering stage, while the mustache variety Kharkivsky etalon was 8.9% less. The specified dependence was observed stably. Meanwhile, the process of differentiation deepened, depending on the studied types of bacterial and nitrogen-phosphorus fertilizers. It was established that the number of nodules on the roots of peas gradually increases starting from the stage of the third leaf, reaching a maximum during the flowering stage, and then gradually decreases until the stage of grain maturation (Fig. 1, 2).

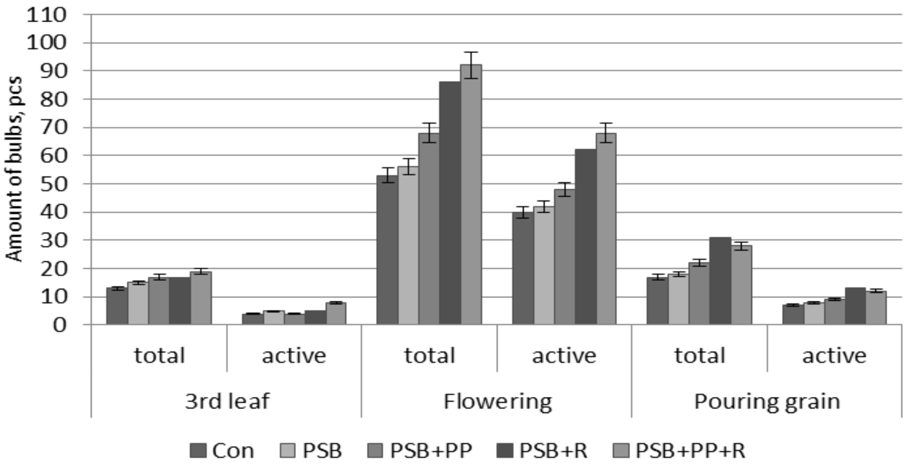



Fig. 1. Change in the number of nodules on the roots of Kharkivsky yantar variety depending on the use of bacterial fertilizers, pcs./plant

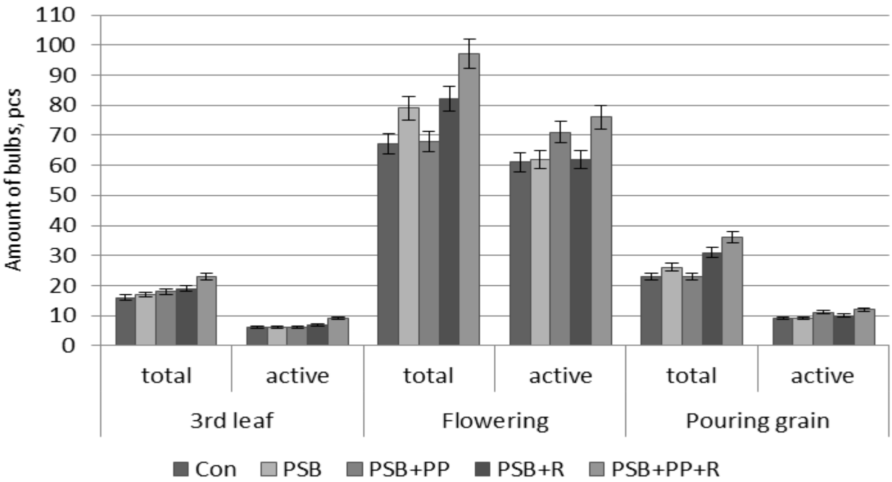



Fig. 2. Change in the number of nodules on the roots of Kharkivsky etalon variety depending on the use of bacterial fertilizers, pcs./plant

There were no noticeable varietal differences in the formation of the number of nodules per pea plant. The total number of nodules was 23 in the stage of the third leaf, and there were 8 active nodules on both cultivars. Their number increased significantly to 93 and 77 pieces to the stage of full flowering respectively. However, during the grain pouring stage, their number decreased due to the cessation of plant growth and development.

The three-component mixture (PSB + PP + Rhizobium) was the most effective in terms of forming the number of nodules on pea roots and provided a significant increase in both the total number of nodules (by 1.4-2.0 times) and their active forms (by 1.3-2.0 times).

The seeds treatment with bacterial inoculants had a greater effect on the yield of peas of both varieties than the effect of pre-sowing mineral fertilizers $N_{20}P_{40}$ (Table 2).

Table 2. The effect of inoculation and mineral fertilizers application on the pea grain yield, t/ha

| Inoculation (factor B) | Without fertilizer (factor C) | | | | $N_{20}P_{40}$ (factor C) | | | |
|---|---|---|---|---|---|---|---|---|
| | Yield, t/ha | | Increment, t/ha | | Yield, t/ha | | Increment, t/ha | |
| | *1** | *2** | *1* | *2* | *1* | *2* | *1* | *2* |
| Control | 1.83 | 1.95 | - | - | 1.93 | 2.10 | - | - |
| PSB | 2.06 | 2.15 | 0.23 | 0.20 | 2.14 | 2.33 | 0.21 | 0.23 |
| PP | 2.07 | 2.21 | 0.24 | 0.26 | 2.13 | 2.35 | 0.20 | 0.25 |
| Rhisobium | 2.03 | 2.17 | 0.20 | 0.22 | 2.13 | 2.35 | 0.20 | 0.25 |
| PSB+PP | 1.99 | 2.19 | 0.16 | 0.24 | 2.12 | 2.33 | 0.19 | 0.23 |
| PSB+R | 1.96 | 2.15 | 0.13 | 0.20 | 2.06 | 2.31 | 0.13 | 0.21 |
| PP+R | 2.01 | 2.20 | 0.18 | 0.25 | 2.10 | 2.35 | 0.17 | 0.25 |
| PSB+PP+R | 2.06 | 2.20 | 0.23 | 0.25 | 2.15 | 2.35 | 0.22 | 0.25 |
| LSD $_{0,95}$ t/ha for factor A | 0.11 | | | | | | | |
| for factor B | 0.09 | | | | | | | |
| for factor C | 0.20 | | | | | | | |
| for interaction ABC | 0.25 | | | | | | | |

*Note: (Factor A): 1* – Kharkivsky yantar; 2* – Kharkivsky etalon*

In particular, the Kharkivsky yantar seeds treatment with bacterial inoculants ensured an increase in the pea grain yield by 0.13-0.24 t/ha (6.6-11.6%) compared to control. Appropriate rhizobial inoculation and fertility management can increase field pea (*Pisum sativa*) seed yield and improve aboveground biomass (Clayton *et al.* 2004). Higher dose of nitrogen fertilizer incorporated at seeding reduced nodule number and $N_2$ fixation process.

The effect of seed inoculation with bacterial strains was 6.5% less in the trial with the introduction of mineral fertilizers. It is known that phosphorus deficiency can restrict the leguminous crops nitrogen fixation (Erman *et al.*

2009). Meanwhile, a single inoculation due to PSB and PP nut led to an increase in grain yield in comparison with control by 0.2-0.26 t/ha (10.2-13.3%). The effect of seed treatment with these bacterial inoculants was less by 0.13-0.22 t/ha (9.2-11.9%) when combined with $N_{20}P_{40}$.

Inoculation with bacterial fertilizers led to an increase in the nitrogen content in the grain of both pea varieties. The increase in phosphorus and potassium was less significant (Figure 3 and 4).

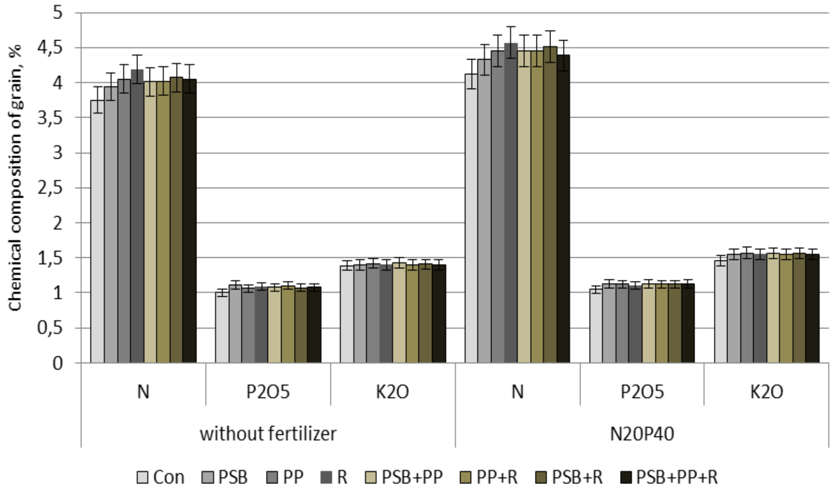



Fig. 3. The effect of inoculation of pea seeds with bacterial fertilizers on the chemical content of the grain (variety Kharkivsky yantar), %

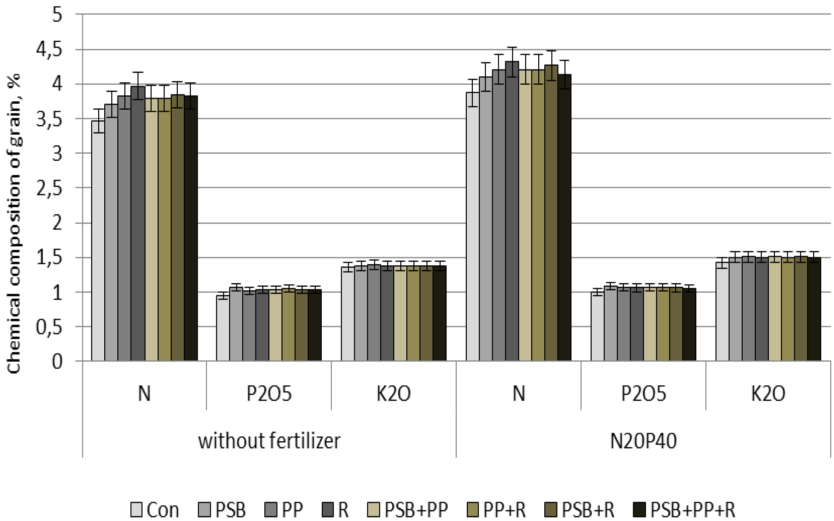



Fig. 4. The effect of inoculation of pea seeds with bacterial fertilizers on the chemical content of the grain (variety Kharkivsky etalon)

The effect of inoculation on the Kharkovsky yantar variety was the same as on the Kharkovsky etalon variety.

It was also revealed a decrease in the content of nitrogen, phosphorus and potassium in the leaf-stalk biomass after inoculation of seeds of both pea cultivars from the beginning of the stage of 6-8 leaves to full maturity (Figure 5, 6). All bio-inoculants contributed to the increase of nitrogen content in leaf-stem biomass of plants in both treatments (without and with fertilizers) from 7 to 21% relative to the control. The highest effect was recorded after inoculation with *Rhizobium leguminosarum* and from the use of double (PSB + PP) and triple mixture (PSB + PP + R) bacterial fertilizers.

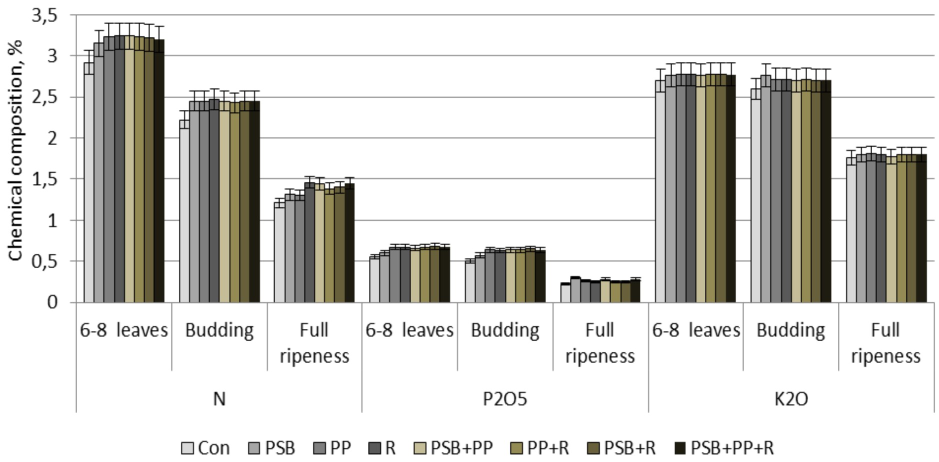



Fig. 5. The effect of pea seeds inoculation of the Kharkivsky yantar variety on the chemical content of leaf-stem mass, % (without mineral fertilizer)

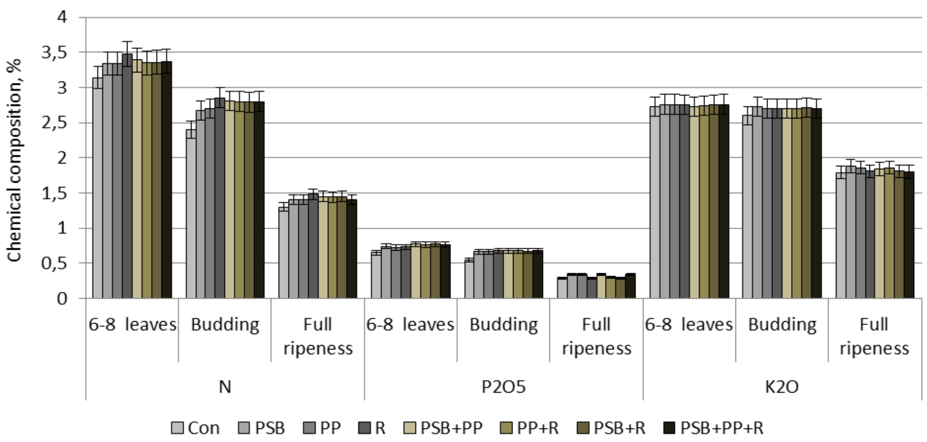
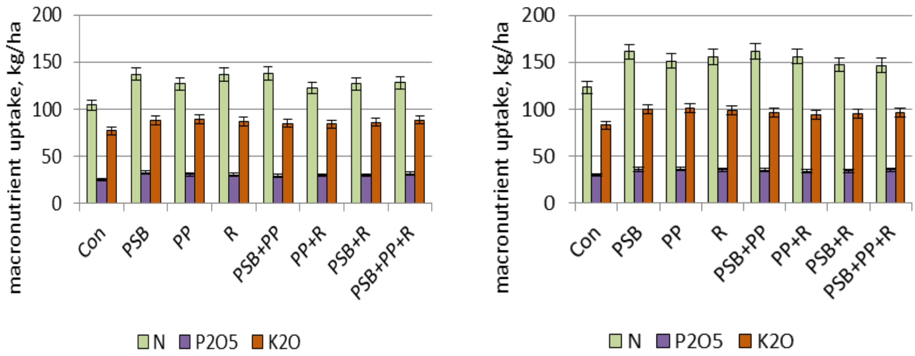



Fig. 6. The effect of pea seeds inoculation of the Kharkivsky yantar variety on the chemical content of leaf-stem mass, % (with mineral fertilizer)

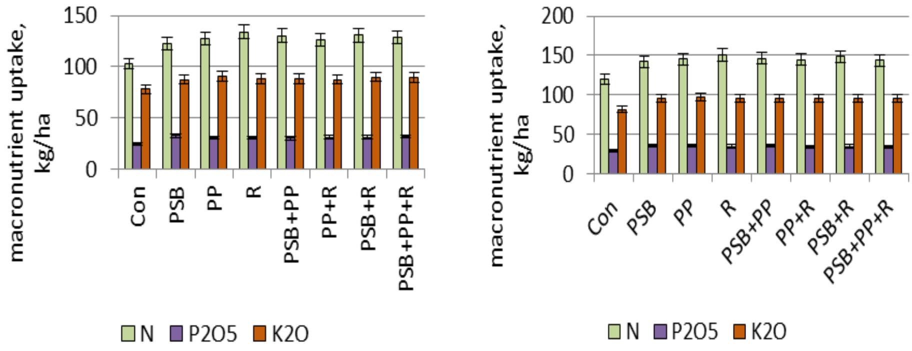
The highest removal of nitrogen in the Kharkivsky yantar variety in the $N_{20}P_{40}$ was with the combination of PSB + PP The amounts of phosphorus and potassium uptake were higher in the PSB version (without fertilizers), and in the treatment with the use of *P.Polymyxa* - with the use of $N_{20}P_{40}$ fertilizers (Figure 7).




*without fertilizers* *with fertilizers*

Fig.7. The effect of inoculation of Kharkivsky yantar pea seeds on nitrogen, phosphorus and potassium uptake with the seed and straw yields, kg/ha

The highest level of nitrogen uptake for to the Kharkivsky etalon variety was observed in the Rhizobium trial, while phosphorus and potassium were observed in the PSB and PP treatments (Figure 8).




*without fertilizers* *with fertilizers*

Fig. 8. The effect of inoculation of Kharkivsky etalon pea seeds on nitrogen, phosphorus and potassium uptake with the seed and straw yields, kg/ha

The uptake of nitrogen, phosphorus and potassium of the Kharkivsky etalon variety with the yield of the main and secondary products was somewhat lower than the Kharkivsky yantar variety. Nitrogen content and accumulation in the plant significantly depend on Rhizobium inoculation and cultivar type (Klimek - Kopyra *et al.* 2018). In other research (Mekonnen and Assefa 2019) Rhizobiium inoculants were effective in accumulation of grain nitrogen content and N uptake (12-14% increases). Probably, the nitrogen content in the grain of the Kharkivsky etalon variety was lower than at the Kharkivsky yantar variety because of their biological characteristics. Therefore, seed inoculation with *Rhizobium leguminosarum* (R) had the maximum effect connected with the nitrogen content increasing in the grain of both cultivars.

## CONCLUSIONS

The largest area of the leaf surface of two pea varieties was formed in the flowering stage in the experiment trials, where a combination of different bacteria strains used for inoculation. Thus, the application of a mixture of three components - FMB + Rhizohumin + PMB in the flowering stage of pea plants contributed to the formation of the leaf surface area of the Kharkivsky yantar cultivar - 48.8, and in the Kharkivsky etalon variety - 47.9 thousand $m^2$/ha compared to the control. Inoculation of peas with phosphorus-mobilizing bacteria contributed to an increase in the leaf surface area compared to the control by 6.5 and 5.6 thousand $m^2$/ha, respectively, or by 13.3 and 11.6%. The three-component mixture (PSB + PP + Rhizobium) seeds inoculation provided a significant increase in both the total number of nodules (by 1.4-2.0 times) and their active forms (by 1.3-2.0 times) on the pea root system. The use of bacterial inoculants for the seed treatment of two pea varieties allowed increasing the grain yield up to 13%. All bio-inoculants contributed to the increase of nitrogen content in the leaf-stem mass of plants on both trials (without and with fertilizers) from 7 to 21% relative to the control. The highest effect (up to 21%) was recorded after inoculation of pea seeds with *Rizobium leguminosarum*. The use of a double (PSB + PP) and triple mixture (PSB + PP + R) of bacterial inoculants provided the second place to an increase in the nitrogen content in the foliar mass of plants in both treatments (without and with mineral fertilizers).

The seeds inoculation with three component mixture (PSB + PP + R) was the most cost-effective and reasonable for the Kharkivsky etalon variety growing in the start fertilization with the rate $N_{20}P_{40}$.

## ACKNOWLEDGEMENTS

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