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THE IMPACT OF THE PEA SEED TREATMENTS WITH BIO-FERTILIZERS AND BIO-AGENTS ON THE LEVEL OF PLANT NITROGEN SYMBIOTIC FIXATION

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Abstract

Main objective was to estimate impact of the pea seed treatments with bacterial fertilizers and bio-agents on the symbiotic nitrogen fixation process in the soil-plant system. Treatments of pea seed with bacterial preparations and bio-agents had different effects chemosynthesis with using nitrification energy derived from reactions involving inorganic chemicals in the rhizosphere of pea. The highest level of soil nitrification energy in the pea rhizosphere was recorded after phosphorus solubilization bacteria (PSB) + Paenibacillus polymyxa (PP) treatment option in the topsoil. The introduction of mineral fertilizers into the soil inhibited the growth of nitrification energy in the pea rhizosphere slightly. The highest pea seeds inoculation effect was observed when phosphorus-mobilizing bacteria (PSB) were combined with Paenibacillus polymyxa (PP). The maximum level of symbiotic nitrogen fixation was noted after pea seed treatments with bio- agent Agat-25 K and PGR Reacom-C.

Key words: *seed treatments; pea varieties; bio-fertilizers; bio-agents; nitrogen symbiotic fixation.*

INTRODUCTION

Field pea grains contain approximately 21-25% protein including high levels of amino acids, lysine and tryptophan, which are relatively low in cereal grains (Mishra et al., 2010). Crop land use change in Ukraine since 2000 has been mainly in favour of industrial crops (oilseed) and within the cereal area, in favour of corn (Fileccia et al., 2014). Such negative phenomena as irrational exploitation, the lack of scientifically based crop rotation, fertilization and protection systems led to a decrease in the area of pea sowing, yield, and protein content from 22.5-23.5 to 19-22% (Lemishko, 2020). The rhizosphere is the area where plant roots and soil composition interact each with other (Chamkhi et al., 2022). During the process of N conversion formed by microorganisms ammonium salt oxidized to nitrate by nitrification (Xiong et al., 2021). The reaction of peas to the availability of nutrients in the soil is closely related to the activity of nodule bacteria. It was established that this crop needs a small amount of nitrogen in the I-III stages of organogenesis, and in subsequent periods the need for nitrogen is replenished due to nitrogen fixation by its nodule bacteria (Jensen, 1987; Date, 2000). It was established that nitrogen fertilizers should be applied taking into account the level of nitrogen symbiotic fixation is 50-70% of the total pea need.

The implementation of various agricultural measures aimed at improving the conditions of nitrogen nutrition leads to an increase in the protein content of grain (Ahmed et al., 2007; Erman et al., 2009; Geneva et al, 2011). Combination of *Nitragina* inoculant with micronutrient fertilization significantly increased root length density compared to control (Klimek-Kopyra et al., 2018). Significant positive correlations between shoot biomass and nodule biomass or number were observed independently of species affiliation and breeding history (Bourion et al., 2018). Dual inoculation of pea plants increased growth, mycorrhizal colonization rate, nodulation parameters, N_2 fixation activity (Stancheva et al., 2006). The maximum number of nodules per plant were recorded where composite culture of Rhisobium+PSB (Tyagi et al., 2003). Meantime, the application of 10 kg N/ha reduced the nodules number mainly because of medium status of organic matter content of soil and low requirement of chemical nitrogen of pea. Pea seeds inoculation led also to increase the number of pods plant^{-1}

by 2 times (Mekonnen and Assefa, 2019). The co-inoculation of Rhizobium + PSB at 40 mg P kg^{-1} soil significantly increased shoot and root length, number of flowers, pod, and nodules per plant, root and shoot dry weight, 100 grain weight and number of grains per pod (Abid et al., 2016). The combined application of *Glomus fasciculatum* and *Rhizobium* also remarkably increased the nitrogen and phosphorus contents of pigeon pea (Bhattacharjee and Sharma, 2012). The results suggest that dual inoculation of *Glomus fasciculatum* and *Rhizobium* revealed synergistic effect to enhance the chlorophyll, nitrogen and phosphorus contents of pigeon pea. The increasing need to stipulate seeds germination, to control plant pathogens and insects in organic agriculture promotes the search for safe and effective compounds from natural sources, especially plant-derived compounds (Chang et al., 2022). Effect of two plant growth stimulators: bactozol (bacterial origin) and D1 (synthetic analog of phytohormones) on metabolism of pea rhizobia (*Rhizobium leguminosarum* bv. *viciae* 2636) and efficiency of their symbiosis with pea plants have been studied (Kosenko et al., 2001). Treatment of pea plants by bactozol (0.1%) increased the efficiency of their symbiosis with pea rhizobia, evoking the growth of the overground and root mass of the plants, quantity of nodules and their nitrogenase activity.

Main objective of this case study was to estimate impact of the pea seeds treatment with bacterial fertilizers and bio-agents on the symbiotic nitrogen fixation process in the soil-plant system.

MATERIALS AND METHODS

The series of field experiments with bio-fertilizers, bio-stimulants and bio-fungicides 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 dose 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 varieties Kharkiv yantar and Kharkiv etalon.The distribution of precipitation by month is extremely uneven. The average annual rainfall is 455 mm. The content of humus in the top of black soil is 4.5%. 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 200 g/ton of seeds; *Bacillus* megaterium phosphorus solubilization bacteria (PSB) - 455 ml/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. Pea seeds before sowing were treated also with biological preparations of plant growth, fungicidal and insecticidal action. Emistym S is the complex of growth substances in a water-alcohol solution, which includes phytohormones of auxin, cytokinin, and gibberellin nature, trace elements, carbohydrates, and fatty acids. These substances complex is made from derivatives of the metabolism of epiphyte fungi, obtained from the roots of medicinal plants. Agate-25K is the multifunctional bio-agent of fungicidal action with plant growth action. The active substance is inactivated bacteria (titre 5-8x1010 cells / ml before inactivation) *Pseudomonas aureofaciens* N 16 and products of their metabolism. Mikosan-N fungicidal activity based on extracts of mushroom glucans (Melo et al., 2020). Reacom-C-beans action is based on chelates of essential trace elements (Cu, Zn, Mo, Mn, Co, B) in a biologically active and plant-available form. The composition of the PGR Vympel - K includes polyatomic alcohols - up to 300 g/l, humic acids - 30 g/l and carboxylic acids of natural origin - 3 g/l (Katelevsky et al., 2020). It is a typical plant growth regulator with adhesive, adaptogenic and cryoprotective effect. Ganol is extract of wormwood (*Artemisia* spp. L.) to use it as product against some seed-borne pathogens in substitution for the chemical pesticides

(Dadasoglu et al., 2015). Its antiseptic action allows for the reduction of damage to plants by phytopathogenic organisms and limits the population of pests at all stages of development. The nitrogen concentration in plant samples was estimated using Kjeldahl method. The coefficient of nitrogen fixation or a number that shows the amount, the ratio of nitrogen from its total removal with the crop, which falls on the fate of fixation by nodule bacteria by peas was determined in the variety without fertilizers (Scherbakov and Rudai, 1983). Data base 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 DISCUSSIONS

Inoculation of pea seeds with bacterial fertilizers had different effects chemosynthesis using nitrification energy derived from reactions involving inorganic chemicals in the rhizosphere of pea variety Yantar (Figures 1 and 2).

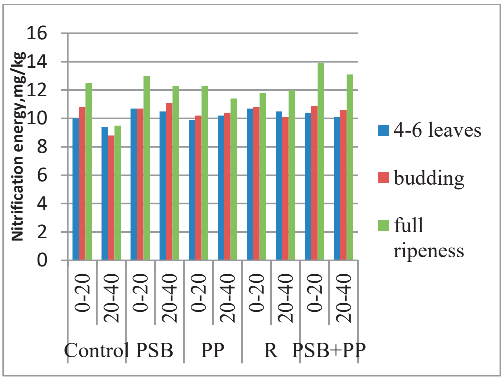

Figure 1. Nitrification energy of soil after bio-fertilizers application (without mineral fertilizers)

The increase in the rate of soil nitrification in the trial without the application of mineral fertilizers continued until the stage of full ripeness. The highest level of soil nitrification energy in the pea rhizosphere was recorded in the PSB+PP treatment option in the topsoil (0-20 cm).

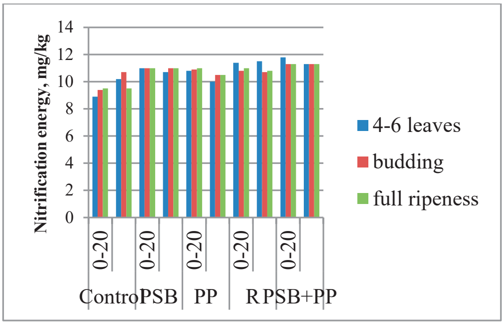

Figure 2. Nitrification energy of soil after bio-fertilizers application (with mineral fertilizers)

Growth of soil nitrification rates in the trial with the initial application of mineral fertilizers ended in the first two stages (4-6 leaves and budding). The highest level of soil nitrification energy in the pea rhizosphere was recorded also in the PSB+PP treatment option in the topsoil. However, the introduction of mineral fertilizers into the soil inhibited the growth of nitrification energy in the pea rhizosphere slightly. It is well known that one of the sources of replenishment of nitrogen reserves in the soil is the biological fixation of atmospheric nitrogen by nodule bacteria, which develop better in soils with a neutral reaction of the environment with a sufficient supply of humus and mobile forms of phosphorus and potassium(Caldwell, 2005). Knowing the dynamics of nitrogenase activity in the root zone of plants grown on different fertilization backgrounds (from deficit to excess nitrogen in the soil) the physiologically justified from crop standpoint doses of mineral nitrogen that will not reduce the nitrogen fixation activity can be determined (Ladha et al., 1986). Meantime, the application of 10 kg N/ha reduced the nodules number mainly because of medium status of organic matter content of soil and low requirement of chemical nitrogen of pea (Tyagi et al., 2003). Determining the dimensions of biological nitrogen fixation is of great importance in solving the problem of increasing soil fertility and agricultural productivity in general. Therefore, biological nitrogen fixation makes corrections when studying the cycle and balance of nitrogen in pea crops. The amount of nitrogen fixation depends on the type of leguminous crop (Posypanov and Knyazeva, 1975). Alfalfa with high hay yields fixes up to

150 kg/ha of nitrogen in the soil, and peas - up to 40-100 kg/ha, depending on the level of the harvest. In favorable years, the coefficient of nitrogen fixation is 0.65 (Scherbakov and Rudai, 1983). The coefficient decreased to 0.59 when phosphoric fertilizers (P_{60}) were applied, and to 0.53 on the background of $P_{60}K_{60}$. The coefficient of nitrogen fixation with rate $N_{60}P_{60}K_{60}$, was minimal and amounted to 0.34. The coefficient of nitrogen fixation by pea nodule bacteria in our experiments was 0.5. The use of phosphorus-mobilizing bacteria for the inoculation of Kharkiv Yantar seeds on a background without mineral fertilizers contributed to an increase in nitrogen fixation on 32% (Table 1). The growth of nitrogen fixation in the dual treatment R+PP was 11.4 and 13.2 kg/ha, or 22 and 25%, respectively. The highest nitrogen fixation was observed when phosphorus-mobilizing bacteria were combined with *Paenibacillus polymyxa* (17.1 kg/ha and 33.0%). Phosphorus-mobilizing bacteria in their combination with *Paenibacillus polymyxa* acted best under the conditions of application of mineral fertilizers. The increase in nitrogen fixation, compared to the control, was at the level of 19.0 and 19.3 kg/ha, or 31%. The overall dimensions of nitrogen fixation were slightly lower in the Kharkiv etalon variety on both growing backgrounds than in the Kharkiv yantar variety. However, the trend of the effect of seed inoculants remained. This result confirms earlier data that the *Paenibacillus polymyxa* can be efficiently used together with phosphorus amendment (Lal and Tabacchioni, 2009).

The Yantar variety pea seeds treatment with bio-agents at the background without mineral fertilizer application contributed to an increase in the nitrates content in the soil rhyzosphere compared to treating seeds with control in the stage of 4-6 leaves (Figure 3). The increase in nitrate content was 19% after seeds treatment with Agat-25K, Emistym-C - 10%, and Reacom - 8% in the trial without mineral fertilizers. Vitavax 200 FF (5%) and Vympel (6%) action was less effective. The above dependencies were also observed in the next stages (budding and full maturity). The nitrogen content of nitrates in the background with the use of mineral fertilizers was 8-20% higher than without fertilizers (Figure 4). At the same time, the seeds treatment action with bio-agents remained the same as on the background without the application of mineral fertilizers. The nitrogen content of nitrates in the background with the use of mineral fertilizers was 8-20% higher than without fertilizers. At the same time, the action of incrustation with biological preparations remained the same as on the background without the application of mineral fertilizers.The effect of seed treatment with PGR and bio-agents was manifested at the determined energy of nitrification after seven days of composting.

The maximum level of symbiotic nitrogen fixation was noted after seeds treatment with bio-agent Agat-25 K and PGR Reacom-C (Table 3). The dimensions of symbiotic fixation of the Kharkiv yantar variety after seeds treatment with Reacom-C and Agat-25 K on the background without the use of fertilizers were 62.6 and 64.5 kg/ha, or 22 and 26% more than in the control (treatment with water). The introduction of mineral fertilizer in a minimum dose of $N_{20}P_{40}$ provided better conditions for the formation of nodules and increased nitrogen accumulation with slightly lower rates of symbiotic nitrogen fixation (13.6 and 24.6%). The dimensions of nitrogen fixation of Kharkiv etalon peas were somewhat lower than those of Kharkiv yantar in all trials of the field experiment. Indicators of nitrogen fixation for the Kharkiv etalon variety in the control without mineral fertilizers were 49.4 kg/ha, in the background with mineral fertilizers - 54.7 kg/ha.

The effect and ratio of indicators for the bio-stimulators and bio-agents remained the same as for the Kharkiv yantar variety. The bio-agent Agat-25 K and PGR Reacom - C beans were most effective, under the influence of which the size of nitrogen fixation on the background without fertilizers increased compared to the control by 23 and 21%, and on the background of mineral fertilizers - by 27 and 34%. A positive effect after processing pea leaves on growth indicators, yield and the number of nodules with biological preparations with a growth-regulating effect was recorded in experiments with garden pea (*Pisum sativum* var Hortense) cv. Pusa Pragati (Syngh et al., 2018).

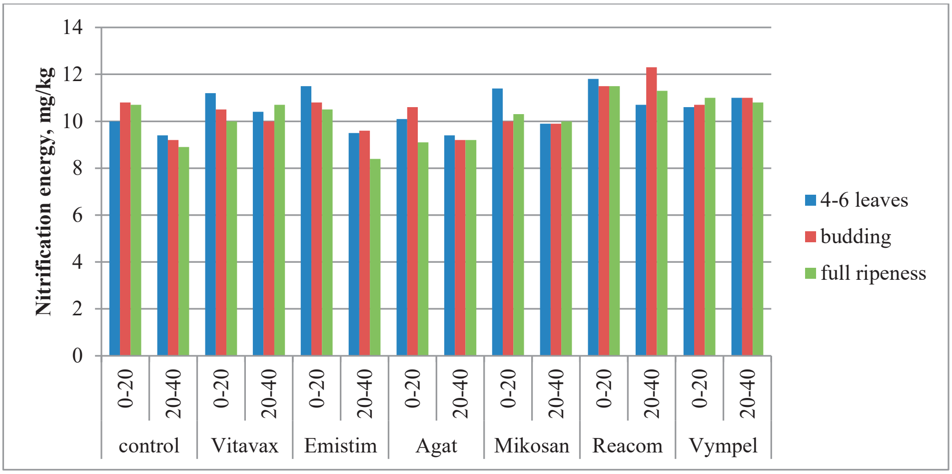

Figure 3. Nitrification energy of soil after bio-stiulators and bio-agents application

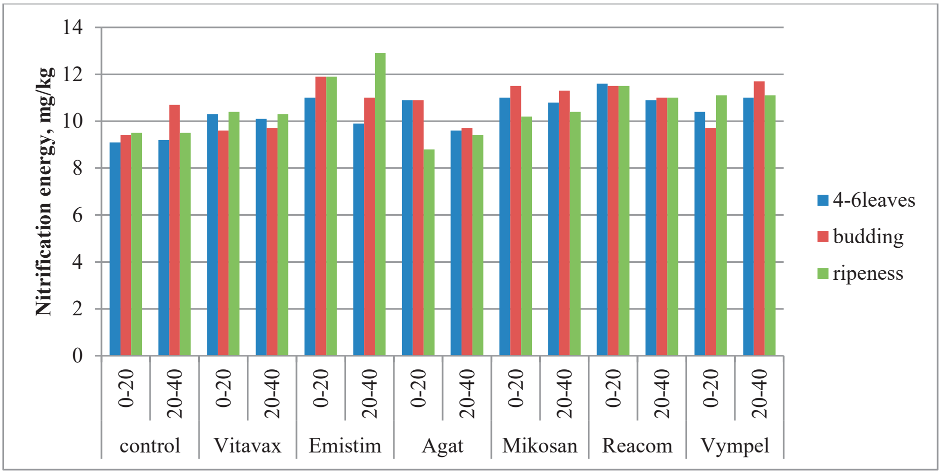

Figure 4. Nitrification energy of soil after bio-stiulators and bio-agents application (with mineral fertilizers)

Table 1. The effect of inoculation of pea seeds with bacterial fertilizers under different feeding conditions on symbiotic nitrogen fixation, kg/ha

Treatment (factor B)	Kharkivsky yantar (factor A)		Kharkivsky etalon (factor A)	
	without fertilizer (factor C)	$N_{20}P_{40}$ (factor C)	without fertilizer (factor C)	$N_{20}P_{40}$ (factor C)
Control	52.0	61.6	51.0	58.8
PSB	68.5	80.6	65.0	69.2
PP	63.4	75.7	62.4	71.1
Rhizobium leguminosarum (R)	65.2	77.9	64.1	73.7
PSB + PP	69.1	80.9	63.5	71.3
PSB + R	66.1	78.1	61.6	70.5
PP + R	63.4	73.8	60,8	71.6
PSB + PP + R	64,0	73.2	62.8	70.5
LSD $_{0,95}$ kg/ha				
factor A	4.10			
factor B	4.41			
factor C	5.70			
combination of ABC factors	6.01			

Table 2. Symbiotic nitrogen fixation depending on the treatment of pea seeds with bio-agents, kg/ha

Tretament (Factor B)	Kharkiv yantar (factor A)		Kharkiv etalon (factor A)	
	Without fertilizers (factor C)	$N_{20}P_{40}$ (factor C)	Without fertilizers (factor C)	$N_{20}P_{40}$ (factor C)
Water (control)	51.4	62.3	49.4	54.7
Vitavaks-200	58.9	65.7	57.3	63.8
Emistim-C	60.0	70.5	59.2	66.3
Agat–25 K	64.5	70.8	60.4	69.9
Mikosan-N	60.0	69.9	56.0	66.0
Reacom-C	62.6	77.6	59.6	73.0
Vympel	57.6	64.5	55.6	62.3
Ganol	59.2	67.2	56.6	64.0
LSD $_{0,95,}$ kg/ha				
for factor A	3.30			
for factor B	2.90			
for factor C	5.51			
for ABC	6.62			

The results of determining the protein yield after seeds inoculation of both varieties with biological fertilizers (without and with the use of mineral fertilizers) are shown in Figures 5 and 6.

Application of mineral fertilizer with a small dose of $N_{20}P_{40}$ provided the highest yield of protein after seed treatment with *Rhizobium*.

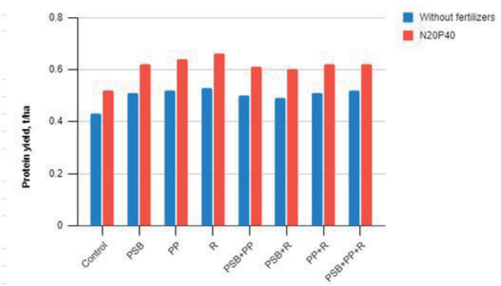

Figure 5. Protein yield of the Kharkiv yantar variety under the influence of seed inoculation with bacterial fertilizers

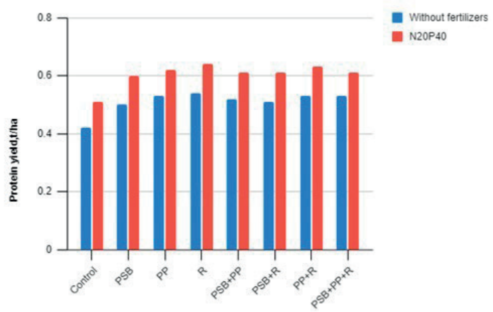

Figure 6. Protein yield of the Kharkiv etalon variety under the influence of seed inoculation with bacterial fertilizers

The results of determining the protein yield after seeds treatments with bio-stimulators and bio-agents of both varieties (without and with the use of mineral fertilizers) are shown in Figures 7 and 8.

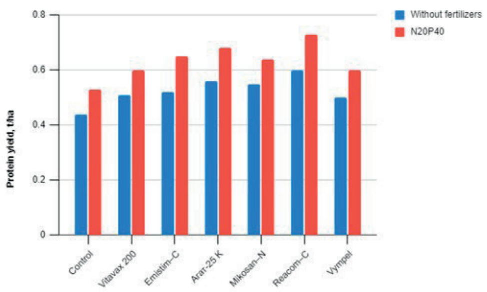

Figure 7. Protein yield of the Kharkiv yantar variety under the influence of seed treatment with bio-stimulators and bio-agents

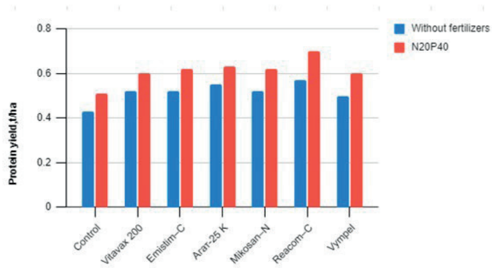

Figure 8. Protein yield of the Kharkiv etalon variety under the influence of seed treatment with bio-stimulators and bio-agents

The highest protein yield was obtained after seeds treatment with Agat-25 K and Reacom-C when $N_{20}P_{40}$ fertilizer was applied.

CONCLUSIONS

Inoculation of pea seeds with bacterial preparations had different effects chemosynthesis with using nitrification energy derived from reactions involving inorganic chemicals in the rhizosphere of pea variety Yantar. The increase in the rate of soil nitrification in the trial without the application of mineral fertilizers continued until the stage of full maturity. The highest level of soil nitrification energy in the pea rhizosphere was recorded at the phosphorus solubilization bacteria (PSB+PP) and *Paenibacillus polymyxa* (PP) treatment option in the topsoil (0-20 cm). The increase in soil nitrification rates in the trial with the initial application of mineral fertilizers ended in the first two stages (4-6 leaves and budding). The increase in nitrogen fixation, compared to the control, was at the level of 19.0 and 19.3 kg/ha, or 31%. The overall dimensions of nitrogen fixation were slightly lower in the Kharkiv etalon variety on both backgrounds than in the Kharkiv Yantar variety. Meanwhile, the trend of action of pea seed inoculants was maintained. Higher costs of using nitrogen, phosphorus and potassium from the soil for the formation of 1 ton of grain were observed with the use of phosphorus solubilization bacteria. Therefore, the pea symbiotic fixation can be improved by using bacterial fertilizers. This approach is much cheaper and easily introduced into the technological cycle of growing this grain crop. It is most appropriate to use for inoculation *Rhizobium leguminosarum* and its combination with PSB+PP. Pea seed treatments with bio-agents Reacom and Agat 25 were the most effective for symbiotic nitrogen fixation.

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