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Evaluation of the effect of rhizobacteria strains application on common bean cultivation under the conditions of Haplic Vertisol and Alluvial-meadow soil

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Abstract: The development of good agricultural practices related to nutrient management and fertilization is important given the increase of greenhouse gas emissions and the pollution of underground waters and rivers by unabsorbed nitrates and phosphates. Microorganisms from the *Rhizobium, Bacillus, Azotobacter,* and *Azospirillum* improve the supply of nutrients in the soil through natural processes of nitrogen fixation, phosphorus and potassium uptake, and production of plant hormones. In this study, an experiment with common bean seeds of the cultivar "Obraztsov Chiflik 12" treated before sowing with selected strains of rhizobacteria from the genus Rhizobium and Bacillus was conducted under the conditions of Haplic Vertisol (HV) and Alluvial-meadow (AM) soil types. The strains and their combinations were evaluated in terms of their effectiveness on the diversity and activity of microorganisms in the soil. The levels of macroelements in the soil and plant biomass were measured. The influence of bacterial inoculation on biometric indicators for improving plant growth was monitored. The results demonstrated the positive effect of pre-sowing seed treatment of common bean with a fresh bacterial suspension of the selected rhizobacterial strains, applied individually or in combination. The effect of the given bacterial inoculant is strongly related to the type of soil. Taken together, the combined agrochemical, microbiological, and biometric data indicate that the selected rhizobacterial strains have potential as biofertilizers/biostimulants to improve common bean cultivation and substitute the mineral fertilization under suitable soil conditions.

Keywords: *Phaseolus vulgaris*; biological nitrogen fixation; rhizobacteria; biofertlizers; PGPR; microbial fertilizers

INTRODUCTION

One of the main sources of pollution on a global scale is the over-application of mineral fertilizers in agriculture. Their use is responsible for the negative effects associated with structural degradation of the soil, reduced fertility, accumulation of heavy metals, and pollution of surface and groundwater (Ma et al. 2024). The increase in greenhouse gases is another negative aspect of the nitrogen fertilizers application. The ecological approaches of replicement of synthetic preparations, chemical fertilizers and pesticides with biofertil-

izers and biological control agents could improve the sustainability of agroecosystems (Kundan, 2015; Oleńska et al. 2020).

Phaseolus vulgaris (common bean) is a traditional crop in Bulgaria. It is one of the representatives of legumes with high nutritional value. Common beans exceed the cereals by 2 to 3 times in protein content. The common bean is rich in fibers, minerals, and starch and is superior to rice in vitamin content (de Almeida Costa et al. 2006).

Phaseolus vulgaris enter into symbiotic relationships with soil microorganisms, which allows plants to assimilate atmospheric nitrogen. It is defined as a non-specific host because enters into symbiosis with different strains of microorganisms, which is the reason for the weak level of nitrogen fixations (Martínez-Romero, 2003). The selection and application of effective rhizobacteria is the way for compensation of that phenomenon.

Plant growth-promoting rhizobacteria (PGPR) include representatives of the symbiotic genera *Rhizobium* and nonsymbiotic *Pseudomonas, Bacillus, Azospirillum, Azotobacter, Klebsiella, Enterobacter,* and *Xanthomonas* (Zhang et al., 2024). They interact with plant roots, stimulating nutrient uptake through multiple mechanisms such as nitrogen fixation, phosphorus and potassium uptake, and the production of plant hormones, which leads to the stimulation of crop growth (da Cunha et al., 2024, Li et al., 2024). On the other hand, there are publications demonstrating the synergetic positive effect of the application of *Rhizobium* bacteria and other PGPR on nodulation level, nitrogen assimilation and yield of several legume crops, including *Phaseolus vulgaris* (Abd El and Azeem, 2022). It has been found that the application of PGPR provides a longer-lasting positive impact on soil fertility compared to untreated (Adesemoye et al., 2009; Bertola et al., 2019; Ullah et al., 2019a; Murgese et al., 2020; Fasusi et al., 2021).

In our study related to the genetic identification of rhizobacterial strains isolated from legumes, including *Phaseolus vulgaris*, high genetic similarity to the genus *Rhizobium* – strain 64.1, and to the genus *Bacillus* – strains 65.2 and 66.2 was found (Metodieva et al., 2026). The effectiveness of strains 64.1, 65.2 and 66.2 isolated from *Phaseolus vulgaris* was tested under field conditions in the region of western Bulgaria (Metodieva et al., 2026). The aim of the present study was to determine the effectiveness of 64.1, 65.2 and 66.2 strains by individual or combined application under the conditions of two soil types: Haplic Vertisol and Alluvial – meadow. The influence of soil and inoculant type on microbiological and biometric indicators related to plant growth was monitored.

MATERIALS AND METHODS

Pot experiment

In the spring of 2024, an experiment was conducted in the vegetation house of the Institute of Soil Science, Agrotechnology and Plant Protection „Nikola Pushkarov“ (ISSAPP). Two soil types, Alluvial-meadow (AM) and Haplic Vertisol (HV), were used. Ten varieties of treatment were applied and described in Table 1. For the soil treatment in variant № 2, a mineral fertilizer (Terra Life, NPK -15-15-15) was applied. Respectively, in variant № 10 the soil was mixed in a ratio of 1/1 with biohumus (biofertilizer from red Californian earthworm without additives). For the treatments № 6, 7, 8, and 9, the strain's culture was mixed equally. All bacterial cultures were with a concentration of 10^8 CFU.

Table 1. Variants of treatment application

Variant	Treatment description
1	Control (no inoculation, no fertilization)
2	NPK (15–15–15), soil application
3	Strain 64.1 (*Rhizobium* sp.), seed inoculation
4	Strain 65.2 (*Bacillus* sp.), seed inoculation
5	Strain 66.2 (*Bacillus* sp.), seed inoculation
6	64.1 + 65.2 + 66.2, seed co-inoculation
7	64.1 + 65.2, seed co-inoculation
8	65.2 + 66.2, seed co-inoculation
9	64.1 + 66.2, seed co-inoculation
10	Biohumus, soil application (1:1, v/v)

The experiment was conducted under natural photoperiodic conditions, without additional artificial light, at uncontrolled but close to outdoor temperatures. Soil moisture was maintained at 60% by regular watering. Plastic pots with a volume of 3 L were used, containing approximately 2.8 kg of air-dry soil. The total number of pots was 120 (10 treatment variants × 6 pots per variant × 2 soil types). Three to four seeds of the common bean variety "Obraztsov Chiflik 12" were sown in each pot, and after germination the plants were reduced to three evenly developed plants per pot (total of approximately 360 plants). Before sowing, the seeds were inoculated with fresh (overnight) bacterial culture from the strains 64.1, 65.2, and 66.2. The common bean cultivar "Obrazcov Chiflik - 12", used for our experiment, was developed by the Institute of Agriculture and Seed Science, Ruse, and passes excellent taste, good digestibility, and an average yield of 174 kg/da.

Microbiological and morphological indicators

During the period of active flowering (phase R6), ten individual plants per variant were monitored for the parameters root length and number of nodules. At the end of vegetation (phase R9), the parameters plant height and dry weight of aboveground biomass were measured as well. Microbiological activity was assessed during both growth phases of plants. The counting of the main groups of soil microorganisms - bacteria using mineral nitrogen; ammonifying; nitrifying; mineral N utilizing bacteria; spore-forming bacteria; actinomycetes; and microscopic fungi was conducted (Grudeva et al., 2006). The results are presented as the number of colony-forming units (CFU) contained in 1g of dry soil. The production of CO_2 was determined titrimetrically by Alef and Nannipieri, 1998. Determination of the amount of microbial biomass C was assessed by Anderson-Domsch 1978.

Agrochemical soil analyses

Soil samples were collected before sowing, during active flowering and after harvesting. The following agrochemical analyses were performed: content of mineral nitrogen (NH_4 and NO_3) (Bremner, 1965, 1996); content of mobile phosphorus and potassium - calorimetrically, according to (Ivanov, 1984); content of organic carbon, according to the modified method of Tyurin (Kononova, 1963); pH - potentiometrically in KCI (Arinushkina, 1962).

Macronutrient content in dry aboveground biomass

The macronutrient content in plant biomass (leaves and stems) was carried out during R6 and R9 phases. The following parameters were monitored: N content, according to Kjeldahl (Bremner et al., 1996); content of K and P– according to EPA - Method 3052.

Statistical analysis

The effect of different treatments on the parameters investigated was assessed by applying ANOVA: single factor (Microsoft, Excel 10). The comparison of the mean values (from 10 individual plants) ± SE was calculated. Differences were considered statistically significant at the level of $p < 0.05$.

RESULTS AND DISCUSSION

1. Agrochemical analyses

The agrochemical analyses conducted before sowing showed that the soil type Haplic Vertisol has an acidic pH reaction, while the soil type Alluvial-meadow has a neutral pH. The measured content of organic C, nitrogen and potassium was higher in the HV soil type, while the amount of phosphorus was more than two times higher in AM soil in comparison with HV.

At the end of the growing season, an increase in the content of total nitrogen compared to the control in the AM soil type after the application of the three strains (variant 6) as well as the combination of strains 64.1 and 65.2 was detected (Table 2). The content of phosphorus and potassium, as well as the organic carbon, was also higher in variant 6 in the same soil type. The increased content of phosphorus after treatment

with strain 66.2 confirmed our data obtained under the same soil type in field conditions (Metodieva et al., 2026). At the conditions of HV soil type, the single application of strain 66.2 also showed a positive effect on the total nitrogen and phosphorus contents. It was interesting to note that the nitrogen content is higher in comparison to the control and variants NPK and biohumus in

Table 2. Agrochemical analyses of Alluvial-meadow soil

Variants	pH		$\sum$ N-NH_4+NO_3	P_2O_5	K_2O	Organic carbon
	H_2O	KCl	mg/kg	mg/100g		%
Before sowing						
	7,0	6,2	10,12	17	36	2,12
End of vegetation						
1. Control	6,9	6	10,4	21,8	17,8	1,93
2. NPK	6,8	5,9	17,3	27,3	25,9	2,2
3. 64.1	6,9	5,9	9,8	20,4	20,3	1,94
4. 65.2	7	5	9,2	20,3	19,3	1,71
5. 66.2	7,1	6	9,2	22,6	21,6	1,76
6. 64.1+ 65.2 + 66.2	7	6,1	13,8	24,1	26,3	2,32
7. 64.1 + 65.2	6,9	6,2	12,7	21,6	23,2	2,2
8. 65.2 + 66.2	7,1	6	10,4	22,3	22	1,9
9. 64.1 + 66.2	7	6	10,9	23,8	25,1	2,08
10. Biohumus	7,2	6,3	19	71,8	71,6	3,44

Table 3. Agrochemical analyses of Haplic Vertisol soil type

Variants	pH		$\sum$ N-NH_4+NO_3	P_2O_5	K_2O	Organic carbon
	H_2O	KCl	mg/kg	mg/100g		%
Before sowing						
	5,4	5,1	13,36	6,05	41,4	2,99
End of vegetation						
1. Control	6,5	5,5	19	0,7	36,4	3,01
2. NPK	6,4	5,3	12,7	2	37,9	2,8
3. 64.1	6,4	5,4	17,9	0,8	23	2,74
4. 65.2	6,5	5,5	17,3	1,1	23,2	2,96
5. 66.2	6,6	5,5	**23,6**	0,9	25,4	2,99
6. 64.1+ 65.2 + 66.2	6,6	5,5	13,3	0,9	26,6	2,8
7. 64.1 + 65.2	6,6	5,5	13,3	1,2	22,7	2,9
8. 65.2 + 66.2	6,8	5,7	13,8	0,6	23,8	2,72
9. 64.1 + 66.2	6,8	5,7	12,7	0,6	25,4	2,82
10. Biohumus	6,7	5,7	17,9	4,95	53,7	6,3

HV soil after application of strain 66.2 (Table 3). The values of phosphorus obtained by us in the control pots with the HV soil type are comparable to those reported by Lozanova et al. 2021.

2. Number of the soil microorganisms

The numbers of bacteria groups and microscopic fungi measured in all variants, including the control, show that soil type AM in combination with common bean growing is more favorable for their development in comparison with soil type HV (Tables 4 and 5).

During the active flowering, ammonifying bacteria and those utilizing N were highly abundant by inoculation with a combination of 64.1 with 65.2 strains in the conditions of AM soil type. The combination between strains 64.1 and 66.2 has a significant effect on actynomicete under the same conditions (p=0.004) (Table 4). Single application of strain 66.2 has a significant

Table 4. Abundance of soil microorganisms underthe Alluvial-meadow soil type (the significant values are bolded)

Period	Active flowering					
CFU /g Alluvial-meadow	Ammonifyining	Actynomicetes	Mineral N utilizing bacteria	Microscopic fungi	Spore-forming bacteria	Nitrifying bacteria
Dilution	10^5	10^5	10^5	10^3	10^5	10^5
1. Control	29,25ab	8,00c	27,25a	10,00b	3,4a	33,00e
2. NPK	80,00f	8,75d	32,75ab	15,50d	5,00c	29,00d
3. 64.1	**43,75cd**	6,25b	**37,00bc**	9,25b	3,00a	25,25c
4. 65.2	**41,25c**	5,50b	24,25a	**17,00e**	**8,70f**	26,00c
5. 66.2	25,25ab	5,00b	25,25a	9,75b	**7,17e**	**42,85g**
6. 64.1+ 65.2 + 66.2	**33,50c**	3,25a	**38,00bc**	5,25a	3,90b	22,75b
7. 64.1 + 65.2	**54,25de**	7,25c	**50,50d**	10,00c	**4,85b**	30,50d
8. 65.2 + 66.2	13,50a	3,00a	23,50a	**12,00c**	**5,92d**	16,25a
9. 64.1 + 66.2	**34,75c**	**9,00d**	26,25a	9,75b	**4,57b**	25,75c
10. Biohumus	63,75e	9,75e	32,00ab	10,25c	3,90b	41,9f
LSD p ≤ 0.05	0	0.0040	0.0450	0,1371	0,0300	0,0005
Period	End of vegetation					
CFU /g Alluvial-meadow	Ammonifyining	Actynomicetes	Mineral N utilizing bacteria	Microscopic fungi	Spore-forming bacteria	Nitrifying bacteria
Dilution	10^5	10^5	10^5	10^3	10^5	10^5
1. Control	28ab	5,66a	24,66c	7,00ab	3,93a	49,00c
2. NPK	67,67f	3,67a	17,33ab	12,33dc	4,50b	30,00ab
3. 64.1	28,00ab	5,00a	14,67a	**15,67de**	**5,63c**	**60,00d**
4. 65.2	**36,67c**	**13,67c**	**41,00e**	**14,67de**	3,80ab	41,33bc
5. 66.2	**45,67e**	5,00a	**31,00d**	**16,00e**	3,66a	**61,00d**
6. 64.1+ 65.2 + 66.2	32,67ab	**6,67b**	19,33b	9,33cd	3,93a	38,00bc
7. 64.1 + 65.2	**42,33d**	4,65a	17,00ab	**15,00de**	**4,20b**	**52,67cd**
8. 65.2 + 66.2	28,33ab	4,00a	11,67a	7,67ab	**4,33b**	27,00ab
9. 64.1 + 66.2	**46,00e**	**8,00b**	**30,00d**	7,33ab	3,46a	33,67ab
10. Biohumus	23,67a	4,67a	18,67ab	5,00a	2,00a	39,00bc
LSD p ≤ 0.05	0.0051	0.2242	0.0084	0.0000	0.0039	0.0004

effect on spore-forming bacteria and nitrifying bacteria (p=0.03; p=0.0005). At the conditions of HV soil type, strain combinations have a significant effect on all measured groups except for spore-forming bacteria (Table 5).

At the end of the growing season the group of nitrifying bacteria demonstrated an increase in the abundance in comparison with the active flowering phase. The number of this particular group demonstrated significant elevation following single application of strains 64.1 and 66.2 and the combination of strains 64.1 and 65.2 in the AM soil type, (p=0.0004) (Table 4). This result could be logically explained with the elevated level of total nitrogen measured in AM soil at the end of plant growing. Under the soil type HV, the combination of strains gives has a significant elevation of nitrifying bacteria also (p=0.008) (Table 5).

In the study of Samy Abd El-Malik and Mohamed Abd El-Azeem (2022), the positive correlation between the activity of microorganisms in

Table 5. Abundance of soil microorganisms under the Haplic Vertisol soil type (the significant values are bolded)

CFU /g (Haplic Vertisol)	Ammonifyining	Actynomicetes	Mineral N utilizing bacteria	Microscopic fungi	Spore-forming bacteria	Nitrifying bacteria
Dilution	10^5	10^5	10^5	10^3	10^5	10^5
1. control	19,50b	4,02a	1,82a	2,16a	6,32e	1,09a
2. NPK	23,57c	8,50d	13,8e	7,16b	1,65b	3,93b
3. 64.1	13,22a	4,55b	**9,85d**	3,50a	1,10a	1,63a
4. 65.2	11,52a	3,72a	**8,00c**	3,42a	1,75b	**2,16b**
5. 66.2	19,20b	4,65b	**14,97f**	**8,32b**	1,92b	**3,04b**
6. 64.1+ 65.2 + 66.2	12,00a	**9,25d**	**4,75b**	**6,75b**	1,67b	**17,00c**
7. 64.1 + 65.2	**37,75d**	**20,25f**	**57,25j**	**10,50b**	4,1d	**34,75d**
8. 65.2 + 66.2	**51,5e**	**8,50d**	**23,50g**	**12,00c**	2,47c	**72,75e**
9. 64.1 + 66.2	23,75c	**6,50c**	**16,00f**	**24,50d**	1,02a	**19,00c**
10. Biohumus	98,75f	13,25e	36,50h	9,75b	2,62c	79,00f
LSD p ≤ 0.05	0	0	0	0	0	0
Period	End of vegetation					
CFU /g (Haplic Vertisol)	Ammonifyining	Actynomicetes	Mineral N utilizing bacteria	Microscopic fungi	Spore-forming bacteria	Nitrifying bacteria
Dilution	10^5	10^5	10^5	10^3	10^5	10^5
1. control	21,66c	5,66b	24,66,0a	9,66a	1,66a	35,33c
2. NPK	31,33f	11,00e	25,33c	22,33b	1,00a	72,33g
3. 64.1	15,00a	3,67a	**28,66c**	**11,33a**	2,33ab	35,00c
4. 65.2	**25,00d**	5,33b	19,66b	**18,33b**	1,00a	30,66b
5. 66.2	16,66a	**13,00f**	22,00b	7,66a	3,66b	27,33a
6. 64.1+ 65.2 + 66.2	26,66e	**6,66c**	**26,33c**	10,33a	2,33ab	**38,33e**
7. 64.1 + 65.2	18,33b	**7,76d**	19,67b	7,67a	2,33ab	27,00a
8. 65.2 + 66.2	16,66a	**6,00c**	19,33b	7,00a	2,00ab	**54,00e**
9. 64.1 + 66.2	22,66c	**11,66e**	**30,66d**	**11,33a**	2,33ab	**36,66d**
10. Biohumus	41,00g	7,00d	28,00c	8,33a	2,33ab	41,00f
LSD p ≤ 0.05	0.0	0.0325	0.5772	0.00	0.3691	0.0080

the soil measured as the number of bacteria and dehydrogenase activity, the availability of nutrient reserves in the soil, and the increased yield of common beans after combined application of *Rhizobuim* with *Pseudomonas fluorescens, Serratia marcescens*, and *Micrococcus agilis* was demonstrated.

3. Microbiological activity

The CO_2 production was elevated in all variants, including the control, measured in AM soil type compared to HV (Table 6). This could logically be associated with the higher number of groups of microorganisms recorded in this type of soil. During the plant's active flowering under both soil types, we found a statistically significant increase in CO_2 values measured in all variants of bacterial inoculation compared to the control (Table 6). During the same phase, the highest CO_2 values were measured after treatment with strain 65.2 in both soil types (Table 6). The combined application of the strains also significantly increased the production of CO_2, which was better manifested in soil type AM.

At the end of the growing season, the activity of microorganisms resulting from elevated CO_2 levels was maintained under both soil types. The highest CO_2 values were recorded after treatment with strain 66.2, as well as with the combination of strains 64.1 and 65.2 under the HV type. At the AM soil, strain 65.2 showed the best results both after individual application and in combination with strain 64.1 (Table 6).

The positive effect observed on basal microbiological activity was confirmed by the results from the induced microbiological response. During both phases, induced microbiological responses measured for all variants of treatment demonstrated a statistically significant increase in CO_2 production compared to the control under both soil types (Table 7). In the study of Petкova et al. (2022), the positive correlation between the production of CO_2 and the numbers of ammonifying bacteria and bacteria utilizing mineral nitrogen was demonstrated. The same correlation could be found in results obtained by our experiment.

4. Biometric indicators of plants.

The root length measured by the active flowering was significantly affected following the single application of strain 66.2 in both soil types. The combination of strains 64.1+65.2 on soil type HV also had a significant effect (p=0.045) on this indicator (statistically comparable to that measured for strain 66.2) (Table 8). In our previous study, a positive effect on the root dry weight was observed in alluvial-meadow soil conditions after

Table 6. Basal microbiological activity (CO_2 production) (the significant values are bolded)

mg CO_2 /100g / 24h	Haplic Vertisol		Alluvial-meadow	
Variants / period	Active flowering	The end of vegetation	Active flowering	The end of vegetation
1. Control	0,38a	1,16a	1,15a	1,24a
2. NPK	0,82bcd	1,8abc	1,59bc	1,93b
3. 64.1	**0,53ab**	1,2a	**1,54abc**	**1,68ab**
4. 65.2	**1,04cd**	1,3a	**1,87c**	**2,03b**
5. 66.2	**0,48ab**	**1,97abc**	**1,63bc**	**1,46ab**
6. 64.1+ 65.2 + 66.2	**0,58abc**	1,34a	**1,65bc**	**1,52ab**
7. 64.1 + 65.2	**0,73abc**	**2,92c**	**1,54abc**	**1,91b**
8. 65.2 + 66.2	**1,2de**	**1,65ab**	**1,67abc**	**1,53ab**
9. 64.1 + 66.2	**0,56ab**	1,13a	**1,34ab**	**1,64ab**
10. Biochumus	1,54e	2,53bc	2,32d	3,01c
LSD p ≤ 0.05	0,0004	0,0424	0,0014	0,0009

single treatment of common bean seed cultivar "Obrazcov chiflik 12" with strains 65.2 and 66.2 under field conditions (Metodieva et al. 2026). Silva et al. (2025) demonstrated a superior effect on root growth after co-inoculation with Rhizobium and Azospirillum of different common bean cultivars.

The number of nodules was significantly influenced by all bacterial treatments, with the effect being more pronounced under the variants applied to soil type AM (p = 0.0007) (Table 8). The highest numbers of nodules were observed in the combinations of strain 64.1 with strains 65.2 or 66.2, respectively, on the AM soil type. The individual application of strains 66.2 and 64.1 also resulted in significantly higher nodule formation under this soil type (Table 8). In contrast, in soil type HV, the individual application of strains 66.2 and 64.1 did not increase the nodule number compared to the control (Table 8), which could be explained with the low pH and phosphorus value measured. However, under the HV soil type, plants inoculated with the combination of strains 65.2 and 66.2 produced a statis-

Table 7. Induced microbiological response (microbial biomass C content) (the significant values are bolded)

mg C / 100 g	Haplic Vertisol		Alluvial-meadow	
Variants / period	Active flowering	The end of vegetation	Active flowering	The end of vegetation
1. Control	2,53a	1,49a	2,35a	2,29a
2. NPK	7,38d	4,12b	5,09b	3,31ab
3. 64.1	6,2c	**7,49de**	6,75b	**5,54bcde**
4. 65.2	5,9c	**5,99cd**	7,6b	**5,94cde**
5. 66.2	6,15c	**7,99d**	6,03b	**4,04abc**
6. 64.1+ 65.2 + 66.2	3,64b	**7,74cd**	5,19b	**5,88def**
7. 64.1 + 65.2	9,56e	**7,87d**	5,94b	**7,6f**
8. 65.2 + 66.2	7,62d	4,37bc	7,48b	6,5ef
9. 64.1 + 66.2	7,4d	6,24cd	5,68b	5,71bcd
10. Biohumus	7,74d	4,12b	6,93b	5,2bcd
LSD p ≤ 0.05	0	0	0,0159	0,0059

Table 8. Biometric indicators measured during active flowering (the significant values are bolded)

Soil type	Haplic Vertisol		Alluvial-meadow	
Variants / parameters	Root length (cm)	Number of nodules	Root length (cm)	Number of nodules
1. Control	18,20a	58b	21,0a	57,83a
2. NPK	24,26b	20,67a	21,16ab	78,33bc
3. 64.1	17,75a	60,12b	**24,5abc**	**140,83cd**
4. 65.2	**27,42c**	54b	**30,33cd**	**91bc**
5. 66.2	**30,66d**	57,66b	**34,16d**	**138,33cd**
6. 64.1+ 65.2 + 66.2	20,14a	**87,28d**	**22,0ab**	**161,33de**
7. 64.1 + 65.2	**32,16d**	**93,83d**	**28,0bcd**	**187,5de**
8. 65.2 + 66.2	**27,08c**	**158,33e**	**22,33ab**	**85,5bc**
9. 64.1 + 66.2	**23,83b**	**75c**	**26,5abc**	**180,67de**
10. Biohumus	23,8b	88,6c	28,0bcd	119,5c
LSD p ≤ 0.05	0,045	0	0,0053	0,0007

tically greater number of nodules. Notably, under AM soil conditions, the number of nodules formed after most of the tretments exceeded the nodules developed following biohumus treatment (Variant 10, Table 8). A positive impact on root nodule formation, enzyme activity, and growth and development of perennial pea after inoculation with *Pseudomonas putida*, *P. fluorescens*, and *Bacillus cereus* compared to non-inoculated plants was reported by Tilak et al. (2006). Application of plant growth-promoting rhizobacteria (PGPR) enhances root system development, thereby facilitating more efficient nutrient acquisition and absorption (Bhat et al., 2022; Saghafi et al., 2019; Guan et al., 2023).

At the end of the growing season, plants exhibited superior development under AM soil compared with HV soil across all treatments, concerning the parameters plant height and aboveground dry biomass (Table 9). Most of the treatment under the AM soil type demonstrated a better response of plant height in comparison to the control. Our data show elevation of dry biomass following application of strain 66.2 alone

Table 9. Biometric indicators measured at the end of the growing season (the significant values are bolded)

Soil type	Haplic Vertisol		Alluvial-meadow	
Variants / parameters	Plant height (cm)	Aboveground dry biomass (g)	Plant height (cm)	Aboveground dry biomass (g)
1. Control	65,5ab	1,82ab	69a	2,7a
2. NPK	63abc	4,0d	113,5c	2,54a
3. 64.1	45,33a	1,19a	110bc	3,32a
4. 65.2	31,16a	0,75a	101,33bc	3,9a
5. 66.2	**105cd**	**2,81abc**	**104bc**	5,2a
6. 64.1+ 65.2 + 66.2	**87,5de**	**2,11bcd**	**104,17bc**	2,41a
7. 64.1 + 65.2	**140f**	**4,93cd**	**91,33abc**	2,93a
8. 65.2 + 66.2	85,5bcd	1,57a	86,83ab	2,06a
9. 64.1 + 66.2	67,4bcd	1,38ab	112,5c	3,76a
10. Biohumus	126,8e	6,30e	114,5c	4,02a
LSD $p \leq 0.05$	0	0	0,0065	0,453

Table 10. Macroelements content in plant biomass under Alluvial-meadow soil type

Alluvial-meadow /Period	Active flowering									
Macroelements / Variants	1	2	3	4	5	6	7	8	9	10
K g/kg	17,67a	16,98a	14,62a	17,10a	17,97a	17,23a	15,52a	18,23a	17,52a	25,08b
N%	2,67ab	3,07c	2,07a	2,70ab	**3,00c**	**2,93c**	2,33a	2,50a	2,23ab	1,83a
P g/kg	4,37ab	5,00c	4,35ab	4,36ab	4,90c	4,65ab	3,85a	3,88a	4,30ab	4,70ab
Alluvial-meadow / Period	The end of vegetation									
K g/kg	14,9ab	24,15d	12,86a	16,8abc	18,84bc	15,73abc	17,84bc	19,51c	17abc	31,26e
N%	0,93a	2,23f	0,97a	**1,60cd**	**1,63cd**	**1,03ab**	**1,17abc**	**1,53cd**	**1,47bcd**	1,67d
P g/kg	3,55ab	4,60bc	3,05a	4,07ab	3,91ab	3,94ab	4,22b	3,76ab	3,62ab	5,33c

$p \leq 0,0006$ (N%); $p \leq 0,0256$ (P g/kg)

or in combination with 64.1 at AM soil, but the results are not significant (Table 9). Consistent with our findings, Filipini et al. (2021) demonstrated that inoculation with *Rhizobium tropici*, as well as *Azospirillum brasilense* applied to either seeds or leaves, exerted a synergistic effect, enhancing plant biomass, nitrogen accumulation, seed weight of 100 seeds, and grain yield of common bean under field conditions. In the study by Hadian et al. (2025), the effect of microbial inoculation of pea plants with strains of the genus *Bacillus* on crop productivity was demonstrated. The authors discussed the potential of this type of biofertilization to enhance the sustainability of the agricultural systems under different environmental conditions.

5. Macroelement content in plant biomass

Plant biomass collected at phase R6 shows that plants were able to accumulate a higher amount of nitrogen after seed inoculation with a combination of the three strains, as well as after the individual application of strain 66.2 at AM soil in, comparison with the control plants (Table 10). In HV soil, almost all bacterial treatments resulted in a significant accumulation of nitrogen in the plant biomass at the same stage (Table 11). At the end of the growing season, we observed a general trend of decreased total nitrogen and phosphorus content compared to the flowering stage across all treatments, including the controls under both soil types (Tables 10, 11). A statistically significant increase in nitrogen content was recorded following the application of strains 65.2 and 66.2, individually and in combination, under AM soil conditions (Table 10). This result coincides with the results obtained under the field experiment conducted in 2023 on the same soil type (Metodieva et al., 2026). At the end of the growing season under HV soil, the highest levels of total nitrogen, potassium, and phosphorus were measured after the application of combinations 64.1 and 66.2 (Table 11). One of the effects of plant growth-promoting rhizobacteria (PGPR) is their influence on phosphate solubility. It has been established that representatives from genera *Pseudomonas*, *Bacillus*, and *Rhizobium* can solubilize insoluble forms of phosphate. The release of low-molecular-weight organic acids (such as gluconic acid), as well as phosphatases and phytases, degrades organic forms of phosphate compounds and acidifies the surrounding environment. These acids chelate the cations bound to phosphate (Dhole et al., 2023). Hashem et al. (2019) further noted that *B. subtilis* enhances nitrogen uptake, directly influencing crop growth and development. In the paper of Egamberdiyeva and Höflich, 2004, the inoculation with PGPR improved the nutrient uptake by pea and cotton plants under the conditions of semi-arid soil from Uzbekistan**.**

Table 11. Macroelements content in plant biomass under Haplic Vertisol soil type

Haplic Vertisol / Period	Active flowering									
Macroelements / Variants	1	2	3	4	5	6	7	8	9	10
K g/kg	12,88a	13,71ab	15,65ab	15,88ab	15,83ab	13,69ab	16,40ab	15,08ab	16,66ab	17,84b
N %	1,73a	2,20ab	1,90a	2,07ab	2,03ab	2,17ab	2,37ab	2,63b	2,13ab	2,70b
P g/kg	2,00ab	2,17abcd	2,06abc	2,46bcd	1,87a	2,37abcd	2,66d	2,59cd	2,42abcd	3,98e
Haplic Vertisol / Period	The end of vegetation									
K g/kg	11,56ab	11,89ab	10,58a	12,44ab	11,91ab	12,41ab	17,04cd	15,55bc	20,75d	19,45cd
N %	1.27ab	1,23ab	1,03a	1,33ab	1,27ab	1,10a	1,60bc	1,57bc	1,93c	1,90c
P g/kg	1.86ab	3,14d	1,44a	2,05abc	1,51a	1,40a	1,80ab	1,68ab	2,63bcd	3,00cd

$p \leq 0.0002$ (K g/kg); $p \leq 0.0016$ (N%); $p \leq 0.0185$ (P g/kg)

CONCLUSION

This study confirms the efficacy of Rhizobium and Bacillus strains as promising biofertilizers for common bean *(Phaseolus vulgaris* L.) cultivation, indicating that the success of microbial inoculation is highly dependent on the soil matrix, with distinct patterns emerging across Alluvial Meadow (AM) and Haplic Vertisol (HV) soil types.

• Nutrient Dynamics and Microbial Activity: Inoculation significantly magnified soil total nitrogen (TN) levels. In HV soil, the application of strain 66.2 resulted in TN concentrations exceeding those achieved by both NPK and biohumus treatments. This nitrogen enrichment facilitated a corresponding increase in nitrifying bacterial populations, particularly in AM soil, where levels surpassed conventional mineral fertilization.

• Soil Metabolism: All bacterial treatments induced a statistically significant rise in basal and substrate-induced respiration (SIR). At the end of the vegetative period, SIR values across all inoculated variants were consistently higher than those in the NPK control, signaling enhanced microbial metabolic activity and carbon cycling.

• Plant Phenology and Biomass: Synergistic effects were observed during the flowering stage, where the 64.1 + 65.2 consortia (in AM soil) and individual applications (in HV soil) significantly optimized root architecture and nodulation. Ultimately, plants grown in AM soil achieved superior height and aboveground biomass, correlating with higher nitrogen uptake in plant tissues.

Taken together, the agrochemical, microbiological, and biometric data indicate that alluvial meadow (AM) soil provides more favorable conditions for the alternative cultivation of common bean when biofertilizers/biostimulants are applied. Future studies should evaluate the applied bacterial inoculation strategies under field conditions to confirm their agronomic potential

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

Alef, K., & Nannipieri, P. (1998). Estimation of Soil Respiration with Closed Bottles. – In: MethodsinApplied Microbiology and Biochemistry, *Harcourt Brace and Company Publishers*, 216-217.

Abd El, A. E. M. M., & Azeem, S. (2022). Effects of co-inoculation of rhizobium and plant growth-promoting rhizobacteria on common bean (Phaseolus vulgaris) yield, nodulation, nutrient uptake, and microbial activity under field conditions. *Journal of Soil and Water Sciences*, *7*(1), 13-26.

Anderson, T., & Domsch, K. (1978). A physiological method for the quantative measurement of microbial biomass in soil. *Soil Biology and Biochemistry*, *10*(3), 215 – 221.

Arinushkina, E. V. (1962). Guidelines for Chemical Analysis of Soils. Publishing *House of Moscow University*, Moscow, p. 490 (Ru).

Adesemoye, A. O., & Kloeppe, J. W. (2009). Plant-microbes interactions in enhanced fertilizer-use efficiency. *Applied Microbioogy Biotechnology,*85(1),1-12. doi: 10.1007/s00253-009-2196-0. Epub 2009 Aug 26. PMID: 19707753.

Adesemoye, A. O., & Kloepper, J. W. (2009). Plant–microbes interactions in enhanced fertilizer-use efficiency. *Applied microbiology and biotechnology*, *85*(1), 1-12.

Bertola, M., & Mattarozzi, M. (2019). Sanangelantoni A.M., Careri M., Visioli G. PGPB colonizing three-year biochar-amended soil: towards biochar-mediated biofertilization. *J. Soil Sci. Plant Nutr.* 19, pp. 841–850.

Bhat, B. A., Tariq, L., Nissar, S., Islam, S. T., Islam, S.U., Mangral, Z. I., Ilyas, N., Sayyed, R. Z., Muthusamy, G., & Kim, W. (2022). The role of plant-associated rhizobacteria in plant growth, biocontrol and abiotic stress management. *J. Appl. Microbiol*., 133(5), pp. 2717-2741.

Bremner, J. M. (1996). "Methods of soil analysis: part III. Chemical methods," SSSA Book Series No. 5, *Soil Science of America and American Society of Agronomy*, Madison, WI, USA, American Society of Agronomy Inc. Madison, Wisconsin, USA. pp. 1179–1237.

Bremner, J. M. (1965). Inorganic forms of nitrogen. In: C. A. Black et al. (Eds.). Methods of Soil Analyses. Part 2: Chemical and Microbiological Properties. № 9, Agronomy.

da Cunha, I. D. C. M., da Silva, A. V. R., Boleta, E. H. M., Pellegrinetti, T. A., Zagatto, L. F. G., Zagatto, S. D. S. S., ... & Mendes, L. W. (2024). The interplay between the inoculation of plant growth-promoting rhizobacteria and the rhizosphere microbiome and their impact on plant phenotype. *Microbiological research*, *283*, 127706.

de Almeida Costa, G.E.; da Silva Queiroz-Monici, K., Reis, S. M. P. M., & de Oliveira, A. C. (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry,* 94, 327–330.

Dhole, A. M., Shelat, H. N., Patel, H. K., & Jhala, Y. K. (2023). Evaluation of the Co-inoculation Effect of Rhizobium and Plant Growth Promoting Non-rhizobial Endophytes on Vigna radiata: AM Dhole et al. *Current Microbiology*, *80*(5), 167.

Fasusi, O. A., Cruz, C., & Babalola, O. O. (2021). Agricultural sustainability: microbial biofertilizers in rhizosphere management. *Agriculture*, *11*(2), 163.

Filipini, L. D., Pilatti, F. K., Meyer, E., Ventura, B. S., Lourenzi, C. R., & Lovato, P. E. (2021). Application of Azospirillum on seeds and leaves, associated with Rhizobium inoculation, increases growth and yield of common bean. *Archives of Microbiology*, *203*(3), 1033-1038.

Guan, H., Liu, X., Fu, Y., Han, X., Wang, Y., Li, Q., ... & He, W. (2023). The locoweed endophyte Alternaria oxytropis affects root development in Arabidopsis in vitro through auxin signaling and polar transport. *Journal of Experimental Botany*, *74*(3), 931-944.

Grudeva, V., Moncheva, P., Naumova, Sv., Gocheva, B., Nedeva, Tr., & Antonova-Nikolova, St. (2006). Manual of Microbiology. University Library „St. Kliment Ohridski“, Sofia, p. 355.

Hadian, S., Smith, D. L., & Supronienė, S. (2025). Modulating the plant microbiome: effects of seed inoculation with endophytic bacteria on microbial diversity and growth enhancement in pea plants. *Microorganisms*, *13*(3), 570.

Hashem, A., Tabassum, B., & Fathi Abd_Allah, E. (2019). Bacillus subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress. Saudi Journal of *Biological Sciences* 26(6), 1291–1297. https://pmc.ncbi.nlm.nih.gov/articles/PMC6734152

Ivanov, P. (1984). New acetate-lactate method for determining of available forms of phosphorus and potassium in the soil. *Soil Science and Agrochemistry*, *19*(4), 88–98 (Bg).

Kundan, R., Pant, G., Jadon, N., & Agrawal, P. K. (2015). Plant Growth Promoting Rhizobacteria: Mechanism and Current Prospective. *Journal of Fertilizers & Pesticides*, *06*(02). doi:10.4172/2471-2728.1000155

Kononova, M. (1963). Soil organic matter. Its nature, properties and methods of study. *Academy of Sciences of the USSR*, Moscow, p. 314. (Ru).

Lozanova, V., & Dimitrov, I. (2021). Influence of Agro-Technical Treatments on Some Physical and Agrochemical Parameters of Haplic Vertisols; *Bulgarian Journal of Soil Science*® Volume 6. Issue 1. www.bsss.bg

Li, X.C., Song, K., Chen, B., Jiang, L., & He, Y.W. (2024). Plant Growth-promoting Rhizobacteria: Mechanisms and Perspectives. *Acta Laser Biol*. Sin.,33(3), pp.193-200. 10.3969/j.issn.1007-7146.2024.03.001

Ma, Y., Suo, Y., Qi, H., Tang, F., & Wang, M. (2024). Effects of rhizobium inoculation on rhizosphere soil microbial communities, physicochemical properties, and enzyme activities in caucasian clover under field conditions. *Agronomy*, *14*(12), 2880.

Martínez-Romero, E. (2003). Diversity of rhizobium-phaseolus vulgaris symbiosis: overview and perspectives. *Plant Soil,* 252, pp. 11–23. doi: 10.1023/A:1024199013926

Metodieva, Ts., Radkova, M., Stefanova, K. & Valchovski, H. (2026). Genetic identification and assessment of the beneficial effect of selected rhizobacteria on plant growth and yield of common bean under the field conditions of West Bulgaria. Bulg. J. Agric. Sci., *32*(1), 142–149.

Murgese, P., Santamaria, P., Leoni, B., & Crecchio, C. (2020). Ameliorative effects of PGPB on yield, physiological parameters, and nutrient transporter genes expression in Barattiere (*Cucumis melo* L.) *J. Soil Sci. Plant Nutr*. 20(2), 784–793.

Petkova, G., Nedyalkova, K., Perfanova, I., Kercheva, M., Hristova, M., & Ilieva, Zh. (2022). Microbiological and physical characteristics of skeletal soils used for tobacco cultivation. *Soil science, agrochemistry and ecology*. Sofia: *56* (1), pp. 3-16.

Oleńska, E., Małek, W., Wójcik, M., Swiecicka, I., Thijs, S., & Vangronsveld, J. (2020). Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. *Science of the Total Environment*, *743*, 140682.

Saghafi, D., Ghorbanpour, M., Shirafkan, Ajirloo, H., Asgari, & Lajayer, B. (2019). Enhancement of growth and salt tolerance in Brassica napus L. seedlings by halotolerant Rhizobium strains containing ACC-deaminase activity. *Plant Physiol.* Rep., 24(2), pp. 225-235.

Silva, G. R., Teixeira, I. R., Ferreira, A. B., da Silva, G. C., Rocha, E. C., Santos, F. L. S., ... & Casaroli, D. (2025). Impact of the application of rhizobacteria in bean cultivars: Potential for sustainable management. *Agronomy*, *15*(2), 377.

Tilak, K. V. B. R., Ranganayaki, N., & Manoharachari, C. (2006). Synergistic effects of plant-growth promoting rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*). *European Journal of Soil Science*, *57*(1), 67-71.

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