Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Isolation and Screening of Plant Growth-Promoting Rhizobacteria and Their Synergistic Interaction with Plant Extracts for Enhancing Growth of Winter Wheat

Ausra Babenskiene

ausra.babenskiene@vdu.lt

Vytautas Magnus University

Justina Kaziuniene

Lithuanian Research Centre for Agriculture and Forestry

Skaidre Suproniene

Lithuanian Research Centre for Agriculture and Forestry

Audrius Gegeckas

Vilnius University

Zita Kriauciuniene

Vytautas Magnus University

Article

Keywords: Triticum aestivum L, plant extracts, bacterial isolates, synergy, biostimulant

Posted Date: October 7th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10767593/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Isolation and Screening of Plant Growth-Promoting Rhizobacteria and Their Synergistic Interaction with Plant Extracts for Enhancing Growth of Winter Wheat

Ausra Babenskiene [1], Justina Kaziuniene [2], Skaidre Suproniene [2], Audrius Gegeckas [3,4] and Zita Kriauciuniene [1]

[1] Department of Agroecosystems and Soil Sciences, Faculty of Agronomy, Agriculture Academy, Vytautas Magnus University, Studentu 11, LT-53361 Akademija, Kaunas Reg., Lithuania. ausra.babenskiene@vdu.lt
[2] Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, Akademija, LT-58344 Kėdainiai Reg., Lithuania.
[3] Life Sciences Center, Institute of Biosciences, Vilnius University, LT-10257 Vilnius, Lithuania.
[4] Bioeconomy Research Institute, Agriculture Academy, Vytautas Magnus University, Studentu 11, LT-53361 Akademija, Kaunas Reg., Lithuania.
* Correspondence: ausra.babenskiene@vdu.lt, Tel:+37065290715; zita.kriauciuniene@vdu.lt; Tel:+37068081811

There are known positive effects of plant extracts as well as various micro-organisms on plants and soil, but information on their synergistic effect on plant productivity and resistance to abiotic and biotic stress and soil health is limited. The aim of this study was to evaluate the micro-organisms of the rhizosphere of winter wheat (*Triticum aestivum* L.) from five different regions of Lithuania and to select bacteria capable of fixing nitrogen and solubilising insoluble phosphorus and potassium compounds. To evaluate the antagonistic properties of bacteria against the pathogenic fungi of *Zymeseptoria tritici* and *Fusarium graminearum*, following compatibility tests with selected plant extracts (nettle, nettle + yarrow, willow bark, field horsetail), to assess the synergistic effect on the germination and biometric parameters of winter wheat. In this study, bacterial isolates JON8 were selected, which can fix nitrogen and solubilised phosphorus and potassium compounds, and RUM4, which fixed nitrogen and solubilised phosphorus compounds. After assessing the antagonistic properties against the *Zymeseptoria tritici* and *Fusarium graminearum* fungi, two bacteria were selected: VDU10 and VOK2. Analysis of homology based on RNA sequencing showed that JON8 corresponds most closely to the RNA sequence of the species *Pseudomonas kilonensis*, RUM4 to the sequence of *Pseudomonas piscicola*, VDU10 to the sequence of *Peanibacillus tundrea*, and VOK2 to the sequence of *Bacillus mycoides*. An assessment of the synergistic interaction between bacterial isolates and plant extracts on the germination and biometric parameters of winter wheat revealed that the mixture of *Pseudomonas sp.* RUM4+*Pseudomonas sp.* JON8+nettle+yarrow extract had a synergistic effect on the germination and root length of winter wheat. The mixture of *Pseudomonas s*p. RUM4+*Pseudomonas* sp. JON8+nettle extract did not exhibit any synergistic effect. Both mixtures tended to increase the germination rate and biometric parameters of winter wheat. The results increase the knowledge about the effectiveness of different biostimulants and biostimulant properties.

Keywords: *Triticum aestivum* L, plant extracts, bacterial isolates, synergy, biostimulant.

1. Introduction

The EU's strategies and the objectives of the Green Deal require a 20% reduction in the use of mineral fertilisers and a 50% reduction in the use of chemical pesticides in agriculture by 2030, as well as an expansion of the area under organic farming. In the pursuit of sustainability, it is essential to explore alternative methods [1]. One such option is biostimulants (BIO). BIO is defined as "substances containing substance(s) and/or micro-organisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes with a view to improving nutrient uptake, nutrient use efficiency, resistance to abiotic stress and/or crop quality, irrespective of nutrient levels" [2]. Studies show that biostimulants can not only stimulate plant growth but also inhibit phytopathogens [3]. Such measures include plant growth-promoting rhizobacteria (PGPR) and plant extracts.

Micro-organism-based biostimulants are a key category of sustainable agricultural products, consisting of beneficial micro-organisms which, when applied to seeds, plants or the rhizosphere, stimulate natural processes that promote plant growth, nutrient efficiency and resistance to abiotic stress. PGPR can directly or indirectly promote growth and increase nutrient absorption and stress resistance [4]. Thus, such micro-organisms can directly improve soil structure, enhance plant nutrient uptake (biological nitrogen fixation, phosphate solubilisation, mycorrhizal symbiosis and chelation of microelements) and also enhance phytostimulation through the production of phytohormones, the induction of tolerance, the activation of defence mechanisms and the improvement of soil structure [4, 5] . The use of PGPR in agriculture is promoted as a sustainable and environmentally safe way to maintain soil health and increase crop productivity. Moreover, the use of PGPR in agriculture does not have a negative impact on the ecosystem, ensures food safety and creates sustainable crop production [6]. Integrating PGPR into agricultural practices shows the potential to reduce the need for chemical fertilizers by 20-30%, while maintaining or even improving crop yields and quality. PGPR also contributes to integrated pest control by inhibiting plant diseases through various mechanisms, including the production of pathogen-antagonizing compounds and the stimulation of systemic resistance in plants [7].

Plant extracts, or phytoextracts, are also classified as biostimulants, providing plants with beneficial compounds, including macro- and micro-nutrients, secondary metabolites, vitamins and growth-promoting hormones. The main mechanisms of action are: simulation of physiological processes by increasing photosynthesis, respiration and nucleic acid synthesis; improvement of nutrient absorption and efficiency by modulating the soil microbial community and soil properties; increase of stress resistance by promoting natural plant protection and production of protective compounds such as antioxidants and osmolytes; modulation of the activity of hormones and enzymes by simulating the activity of phytohormones [8, 9]. The use of nettle *(Urtica dioica)* extract inhibits the spread of plant pathogens. It is rich in boron, sulphur, calcium, phosphorus, nitrogen, phenolic compounds, antioxidants and chlorophyll. In addition, nettle extract stimulates the absorption of nitrogen but does not affect the absorption of phosphorus and potassium [10]. The antioxidant, antibacterial, anti-inflammatory and anti-hepatotoxic activity of yarrow (*Achillea millefolium* L.) extract is attributed to its high content of polyphenolic and flavonoid compounds [11]. Willow bark (*Salix spp. cortex*) extract contains salicylic acid, which promotes plant growth and development [12]. It regulates the key physiological processes, including seed germination, flowering and root growth, as well as modulates the plant's response to biotic and abiotic stresses. Willow bark extract improves the plant's ability to thrive under stress, activating defence mechanisms, especially systemic acquired resistance (SAR), which strengthens the plant's ability to resist pests and pathogens. In addition, the willow bark extract promotes flowering by regulating both the autonomic and photoperiodic pathways that control this process [13]. A characteristic of field horsetail (*Equisetum arvense* L.) is that it is one of the plants whose shoots accumulate the highest levels of silicon (Si) [14, 15]. It is beneficial for plant growth by strengthening the plant's cytoskeleton, optimising the photosynthesis process, improving nitrogen fixation and increasing resistance to abiotic and biotic stressors, acting as a trap for reactive oxygen forms (ROS) [16, 17] . Silicon acids also exhibit antifungal activity [18, 19, 20]. The use of field horsetail in agriculture is limited and little studied [16].

The development and application of biological control systems can effectively address two main challenges: Environmental pollution and the development of resistance to chemical fungicides[21, 22]. Effective management of biotic and abiotic stress is essential to ensure high productivity, plant and soil health[9]. The beneficial effects of plant extracts and various micro-organisms on plants and soil are well known; however, information on their synergistic effects on plant productivity, resistance to abiotic and biotic stresses, and soil health is limited [23]. It is argued that research under controlled and

natural conditions, as well as new research methods, is required to substantiate the effects of biostimulants [24, 25]. Harnessing the synergistic effects of plant biostimulants: the transition to a new generation of biostimulants [26].

The aim of this study was to isolate micro-organisms from the rhizosphere of winter wheat *(Triticum aestivum)* in five different regions of Lithuania. To select bacteria capable of fixing nitrogen and solubilising insoluble phosphorus and potassium compounds. To evaluate the antagonistic properties of bacteria against the pathogenic fungi *Zymeseptoria tritici* (the causative agent of leaf septoria) and *Fusarium graminearum* (the causative agent of ear fusariosis), which are among the main pathogens affecting winter wheat. *Zymosseptoria tritici* is the most common disease in Europe, affecting crops, the effect on yield can lead to up to 40% of crop losses and depends on both the intensity of infection and the stage of plant growth during infestation [27]. *Fusarium graminearum* causes significant crop yield losses, and in particular a deterioration in quality due to contamination with various fungal mycotoxins that pose a significant threat to human and animal health [28]. Which, following compatibility tests with selected plant extracts (nettle, nettle + yarrow, willow bark, field horsetail), to assess the synergistic effect on the germination and biometric parameters of winter wheat.

2. Materials and Methods

2.1 Sampling and Isolation of Micro-Organisms

For this study, winter wheat soil rhizosphere samples were collected from 5 test stations in Lithuania: Testing stations of Vytautas Magnus University Academy of Agriculture (VMU) and Lithuanian Research Centre for Agriculture and Forestry: Joniškėlis (Jon), Vėžaičiai (VEZ), Vokė (VOK), Rumokai (RUM). The roots of winter wheat (*T. aestivum*) are pulled out of the soil, and any excess loose soil is removed from the plant roots by gently shaking them. Soil samples from the rhizosphere were stored at 4 °C in sterile 150 ml test tubes prior to the micro-organism isolation procedure [29] .

To isolate soil micro-organisms, 1 g of soil sample is added to 10 ml of sterile deionised water (SDW), and the resulting suspension is incubated in a 100 mL Erlenmeyer flask for 20 minutes. At a temperature of 30 ± 0.5 °C, 130 rpm, in a shaking incubator [29, 30] .

After incubation, the soil suspension is diluted in 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} using the serial dilution method. Soil suspensions of varying concentrations were applied, 100 µL at a time, onto a universal, solid Luria—Bertani (LB) culture medium [10 g tryptone, 5 g yeast extract and 10 g sodium chloride, made up to 1 L with water] [31]. Petri dishes containing soil suspensions are incubated in a bacteriological incubator at 30 °C for 48 hours [30].

After 48 hours of incubation, the number of morphologically different bacteria was visually evaluated. Each morphologically distinct bacterial colony was isolated by subculturing it onto solid LB medium. The first collection of 50 morphologically different bacteria was established.

2.2 Selection of Nitrogen-Fixing Bacteria

The selection of nitrogen-fixing bacteria was carried out in a solid Ashby mannitol medium [0.2 g K_2HPO_4, 20 g mannitol, 0.1 g NaCl, 0.1 g K_2SO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 5 g $CaCO_3$, 20 g agarose and distilled water to make up to 1 L] (Doty et al. 2009) and in semi-solid NFB [0.4 g KH_2PO_4, 0.1 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2$, 0.01 g $FeCl_3$, 0.002 g $MoO_4Na.\ 2H_2O$, 5.0 g sodium malate and 5 ml bromothymol blue in a 0.5% alcohol solution, and 20.0 g agar, water to 1 L, pH 7] [32] in nitrogen-free culture media.

Isolates on Ashby mannitol agar medium were incubated in a bacteriological incubator for 7 days at 30 °C. The growth of bacterial biomass on a nitrogen-free solid Ashby mannitol medium indicates the potential for atmospheric nitrogen fixation.

The nitrogen fixation capacity of these same isolates was assessed in a semi-solid NFB nutrient medium. A single colony of the test bacterium was inoculated into a test tube containing NFB medium. The test tubes with the bacterial colony deposited were incubated in a bacteriological incubator at 30 °C for 5 days. After incubation, the colour of the NFB medium was evaluated. Positive nitrogen fixation is observed if the colour of the medium changes from green to blue. The colour changes are caused by the production of ammonia as the bacteria fix nitrogen. Ammonia is a weak base that changes the pH of the medium. The alkalinity of the culture medium was monitored using bromothymol blue as an indicator[30, 32, 33].

2.3 Screening of Bacteria that Release Insoluble Phosphorus Compounds

The screening of phosphate-solubilising bacteria was carried out using a modified Pikovskaya (PVK) medium [10 g of dextrose, 5 g of tribasic phosphate $(Ca_3PO_4)^2$, 0.5 g $(NH_4)^2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.0001 g $MnSO_4$ and $FeSO_4$, 0.5 g yeast extract and 15 g agar, distilled water to 1 L] solid nutrient medium [34]. The medium contains precipitated

tricalcium phosphate, which phosphate-solubilising bacteria can break down by secreting organic acids; as a result, halos — transparent zones — form in the zones of decomposition [35]. The isolates were aseptically inoculated into the centre of an agar plate. All the plates were incubated in a bacterial incubator at 28 °C ± 2 °C for 14 days. The phosphate solubilisation capacity of each bacterium was assessed in 4 replicates [36]. The diameter of the transparent zones around the colony indicated phosphate solubilisation and was assessed after 2, 5, 7, 10 and 14 days of incubation and measured as the phosphate solubilisation index (PSI) (2.1).

Solubilization Index (PSI) = Colony diameter + Halo zone diameter/ Colony diameter

2.4 Screening for Bacteria Capable of Degrading Insoluble Potassium Compounds

The screening for bacteria capable of solubilising insoluble potassium compounds that are unavailable to plants was carried out on Aleksandrov's solid nutrient medium [5 g dextrose, 0.005 g $MgSO_4$-$7H_2O$, 0.1 g $FeCl_3$, 2.0 g $CaCO_3$, 3.0 g potassium feldspar powder, 2.0 g calcium phosphate and 20 g agar-agar, distilled water to 1 L] [37]. The isolates were aseptically inoculated by spot inoculation in different areas of the Petri dish. The dishes were incubated at 30 °C for 7 days. After 7 days, the effectiveness of potassium dissolution (transparent zones) was evaluated, which was calculated using the Khandeparkar's selection ratio (KSR) [38] (2.2).

Khandeparkar's selection Ratio (KSR) = Diameter of zone of clearance (D) /Diameter of growth (d)

2.5 Evaluation of the Antagonistic Properties of Isolates

The evaluation of the antagonistic properties of the isolates was carried out using two major pathogens prevalent in winter wheat: *Zymoscuttoria tritici* (leaf septoria) and *Fusarium sp. graminearum* (ear fusariosis) [27, 39] . The pathogenic fungi were obtained from the collection of the Microbiology Laboratory at the Lithuanian Research Centre for Agriculture and Forestry.

100 µL of a prepared suspension of the pathogenic fungus was smeared onto the surface of a solid potato dextrose agar (PDA) medium. Using sterile pipette tips, four 5 mm wells were made in a medium containing a fungal suspension; two of these were filled with 100 µL of supernatant from different bacterial cultures, whilst the remaining one was filled with 100 µL of sterile water (control). Each trial was carried out in four replicates [40].

Dishes containing spores of the fungus *F. graminearum* were incubated in a bacteriological incubator at 27 °C in the dark for 5 days [41], dishes containing spores of the fungus *Z. tritici* were incubated in a bacteriological incubator for 10 days in the dark at 18 °C [42]. If, following the incubation period, a clear zone of inhibition forms around the well (where the fungal culture does not grow), this indicates that the bacteria are secreting substances that inhibit the growth of the pathogenic fungi *Z. tritici* and *F. graminearum*. Inhibition of fungal growth was assessed by measuring the zone of inhibition (mm). The attenuation zone was measured in mm, minus the diameter of the well (8 mm) [43].

2.6 Determination of Endospore Formation

The ability of the bacteria to form endospores was assessed microscopically and using the standard serial dilution and agar plating method. First, the bacterial suspension (not less than 1.2 * 10^9 CFU ml^{-1}) was heat-treated at 75 °C whilst incubated in a water bath for 15 minutes. The sample was examined using an OPTIKA B—500BPL binocular laboratory microscope at a magnification of 1,000 times. One millilitre of the heat-treated bacterial suspension was then spread onto a solid LB medium and incubated in a thermostat at 30 °C for 48 hours. Following incubation in a laboratory incubator, the bacterial colonies were counted, and an assessment was made as to whether the colonies that had grown were morphologically identical to those of the bacteria under investigation. If the colonies are identical and numerous, this means that this species of bacterium can form spores [30].

2.7 Preparation of Bacterial Suspensions for Inoculation

To prepare the inoculum, a pure bacterial culture grown no more than a week previously and liquid Luria—Bertani (LB) medium were required. Under sterile conditions, 200 ml of sterile LB culture medium was poured into a 1-litre Erlenmeyer flask, and a single bacterial colony was inoculated using a sterile loop. The flask is sealed and the prepared inoculum is incubated in a laboratory shaker for 24 hours at 30 °C and 130 rpm [29].

Following incubation, the inoculum was spread onto solid Luria—Bertani (LB) medium using the serial dilution agar plating method. The purity of the reconstituted inoculum was then checked, and the number of viable cells (CFU) was evaluated.

2.8 Molecular Identification of Isolated Micro-Organisms

Four isolates which demonstrated the best performance on nitrogen fixation, insoluble phosphorus and potassium compounds solubilization and biological activity against wheat pathogens were identified using partial 16S rRNR sequence analysis. Partial 16S rRNR sequences were determined by PCR, by using primers 8 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). Partial 16S rRNA sequences were identified and compared in the EzBiocloud identification service. Isolates phylogenetic trees were constructed by using MEGA 5.0 software, related type strains 16S rRNR sequences were used for comparison.

Based on the results of the identification tests carried out on the isolated bacteria, the safety class of the isolated bacteria was determined in accordance with the classification set out in the German TRBA 466 (Technical Regulations for Biological Agents)[44].

2.9 Compatibility Study of Bacteria and Plant Extracts

After the results of the study on the identification of isolated bacteria are obtained, the study is continued with 4 different types of bacteria. Bacterial suspensions (inoculants) are cultured.

Each bacterial suspension (at least $1.2*10^9$ CFU ml^{-1}) was mixed with different plant extracts in a ratio of 1:5. In total, four plant extracts were studied. 3 extracts: nettle (*Urtica dioica*), willow bark (*Salix spp. cortex*), field horsetail (*Equisetum arvense*), purchased from the manufacturer J3C Agri, France. 1 extract: nettle and yarrow (*Urtica dioica*+ *Achillea millefolium*) from the manufacturer Schacht, Germany.

The stability of mixtures of different bacteria and extracts was assessed after 1 and 3 hours, after 1 and 2 weeks, and after 1 month, by plating the mixtures onto LB solid medium using the serial dilution and agar coating method [45]. The dishes were incubated in a bacteriological incubator at 30 °C for 48 hours; following incubation, the cell count per 1 ml (CFU ml^{-1}) was determined [46]. Pure bacterial suspensions, not mixed with plant extracts, were used as the control. The stability study was conducted in four repetitions.

2.10 The Effect of Bacterial and Plant Extracts on the Germination and Biometric Parameters of Winter Wheat

Following a study of the compatibility of the selected bacterial and plant extracts, and after assessing the stability of the mixtures, the wheat seeds were disinfected and coated with a mixture of different bacterial suspensions and plant extracts, as well as with each component separately, in accordance with the scheme provided (Table 1).

Table 1. Experimental treatments for the coating of winter wheat seeds.

No.	Code	Treatment	Ratio
1	K	Water	Pure water
2	RUM4	RUM4+Water	1:5
3	JON8	JON8+Water	1:5
4	RUM4+JON8	RUM4+JON8+Water	1:1:5
5	RUM4+JON8+D	RUM4+JON8+*Urtica dioica*+Water	1:1:5:5
6	RUM4+JON8+DK	RUM4+JON8+ (*Urtica dioica*+*Achillea millefolium)* +Water	1:1:5:5
7	D	*Urtica dioica*+Water	5:5
8	DK	*(Urtica dioica*+*Achillea millefolium)*+Water	5:5

Winter wheat seed surface has been sterilised according to standard protocol. The seeds were first immersed in 70% ethanol for 1 minute to remove all external contaminants. Then, for 10 minutes, immersed them in a 2.5 % sodium hypochlorite solution to remove any micro-organisms from the surface. They were then thoroughly rinsed five times with sterile distilled water to remove any remaining traces of ethanol and sodium hypochlorite [47]. The prepared seeds are coated with different mixtures of suspensions, at a rate of 2 litres of mixture per tonne of seed.

Petri dishes are prepared for the experiment, with a single Whatman No. 2 filter paper disc placed in each. The filter paper is moistened with 2 ml of sterile distilled water, and the coated seeds are arranged on it, 25 seeds per Petri dish (400 seeds per treatment). Winter wheat (*T. aestivum*) is germinated in the dark for 7 days, 3 days at 8—12 °C, and then at 20 °C. Germination energy was determined in the laboratory after 3 days and germination after 7 days [48].

Measurements of the above-ground parts and root length were carried out after 7 days by measuring the height of the plants and the length of the roots in mm. The root area, diameter and total length were measured using the Delta-T SCAN root scanning system, and the data were processed using HP Solution Centre software. In addition, after removing the remaining husks, the fresh weight and dry weight of the wheat were measured. To determine the dry matter content, the wheat was dried in a drying cabinet at a temperature of 60 °C for 48 hours, until a constant weight was reached [49]

2.11 Statistics

For the statistical analysis of the data, a three-factor analysis of variance was chosen, using the ANOVA statistical analysis software from the DISVEG package. In this paper, the following letters (a,b,c…) are used to indicate statistically significant differences at confidence levels of 95% and 99%: R_{05} — the margin of reliable difference at a probability level of 95%.

3. Results

3.1. Isolation of Isolates and Description of their Physiological Properties

In this study, samples of the soil rhizosphere of winter wheat (*T. aestivum*) were collected from 5 test stations in Lithuania: Testing stations of Vytautas Magnus University Academy of Agriculture (VMU) and Lithuanian Research Centre for Agriculture and Forestry: Joniškėlis (JON), Vėžaičiai (VEZ), Vokė (VOK), Rumokai (RUM). 50 morphologically distinct isolates were isolated from the soil samples collected, and their plant growth-promoting, antagonistic and morphological properties were examined (Table 2).

Table 2. Plant growth-promoting, antagonistic, and morphological characteristics of the isolates. Growth of isolates in semi-selective media (N_2 fixation assay), no growth (−), weak growth (+), medium growth (++), and intensive growth (+++). Antagonistic activity, Gram staining and endospore formation, positive (+), negative (-).

No.	Isolate	N fixation		Phosphate Solubilization Index (PSI)					Potassium Solubilization	Antagonistic activity		Gram training	Endospore formation
				Days of incubation									
		Ashby's	NFB	2	5	7	10	14	Khandeparkar's selection ratio (KRS)	*F. graminearum*	*Z. tritici*		
1	RUM1	+	-	-	-	-	-	-	-	-	-	+	+
2	RUM2	-	+	-	-	-	-	-	-	-	-	+	+
3	RUM3	+	-	-	-	-	-	-	-	-	-	-	-
4	RUM4	+	+	2.08	2.10	2.16	2.25	2.30	-	+	-	-	-
5	RUM5	++	-	-	-	-	-	-	-	-	-	-	-
6	RUM6	+	-	-	-	-	-	-	-	+	-	+	+
7	RUM7	+	-	-	-	-	-	-	-	-	-	+	+
8	RUM8	-	-	-	-	-	-	-	-	+	-	-	-
9	RUM9	+	-	-	-	-	-	-	-	-	-	+	+
10	RUM10	+++	+	-	-	-	-	-	-	-	-	+	+
11	JON1	-	-	-	-	-	-	-	-	-	-	+	+
12	JON2	-	-	-	-	-	-	-	-	-	-	-	-
13	JON3	+	+	-	-	-	-	-	-	-	-	-	-

14	JON4	+	+	-	-	-	-	-	-	-	-	-	-
15	JON5	+	-	-	-	-	-	-	-	-	-	-	-
16	JON6	+	+	-	-	-	-	-	-	-	+	+	+
17	JON7	+	-	-	-	-	-	-	-	-	-	-	-
18	JON8	++	+	2.08	2.08	2.10	2.10	2.10	2.51	+	-	-	-
19	JON9	+	-	-	-	-	-	-	-	+	-	+	+
20	JON10	+++	-	-	-	-	-	-	-	-	-	+	+
21	VDU1	+	-	-	-	-	-	-	-	+	-	-	-
22	VDU2	-	-	-	-	-	-	-	-	-	-	+	+
23	VDU3	+	+	-	-	-	-	-	1.50	-	-	+	+
24	VDU4	+	-	-	-	-	-	-	-	-	-	+	+
25	VDU5	++	+	-	-	-	-	-	-	-	-	+	+
26	VDU6	+	-	-	-	-	-	-	-	+	+	-	-
27	VDU7	+	-	-	-	-	-	-	-	-	-	-	-
28	VDU8	++	-	-	-	-	-	-	-	-	-	+	+
29	VDU9	+	-	-	-	-	-	-	-	-	+	-	-
30	VDU10	+++	-	-	-	-	-	-	-	+	+	+	+
31	VOK1	+	-	-	-	-	-	-	-	-	-	+	-
32	VOK2	+++	+	-	-	-	-	-	-	+	+	+	+
33	VOK3	+	-	-	-	-	-	-	-	-	-	-	-
34	VOK4	+	-	-	-	-	-	-	-	-	-	-	-
35	VOK5	+	-	-	-	-	-	-	-	-	-	+	+
36	VOK6	+	-	-	-	-	-	-	-	-	-	-	-
37	VOK7	++	+	-	-	-	-	-	-	-	-	-	-
38	VOK8	++	-	-	-	-	-	-	-	-	-	+	+
39	VOK9	++	-	-	-	-	-	-	-	-	-	+	+
40	VOK10	-	-	-	-	-	-	-	-	+	-	-	-
41	VEZ1	+++	+	-	-	-	-	-	-	-	-	-	-
42	VEZ2	-	-	-	-	-	-	-	-	-	-	-	-
43	VEZ3	+	+	-	-	-	-	-	-	-	-	-	-
44	VEZ4	+	-	-	-	-	-	-	-	-	-	+	+
45	VEZ5	-	-	-	-	-	-	-	-	-	-	+	+
46	VEZ6	-	-	-	-	-	-	-	-	-	-	-	-
47	VEZ7	++	+	-	-	-	-	-	-	-	-	-	-
48	VEZ8	+	+	-	-	-	-	-	-	-	-	-	-
49	VEZ9	+++	+	-	-	-	-	-	-	-	-	+	+
50	VEZ10	+	-	-	-	-	-	-	-	-	-	-	-

Studies using semi-selective media revealed that not all isolates were able to grow on nutrient media that did not contain nitrogen. 42 isolates grew on Ashby medium, six of which were characterised by intensive growth: RUM10, JON10, VDU10, VOK2, VEZ1, VEZ9. In NFB medium, 17 isolates formed a biomass ring and changed the colour of the culture medium from green to blue, indicating possible atmospheric nitrogen fixation (Figure. 1). A positive nitrogen fixation reaction was

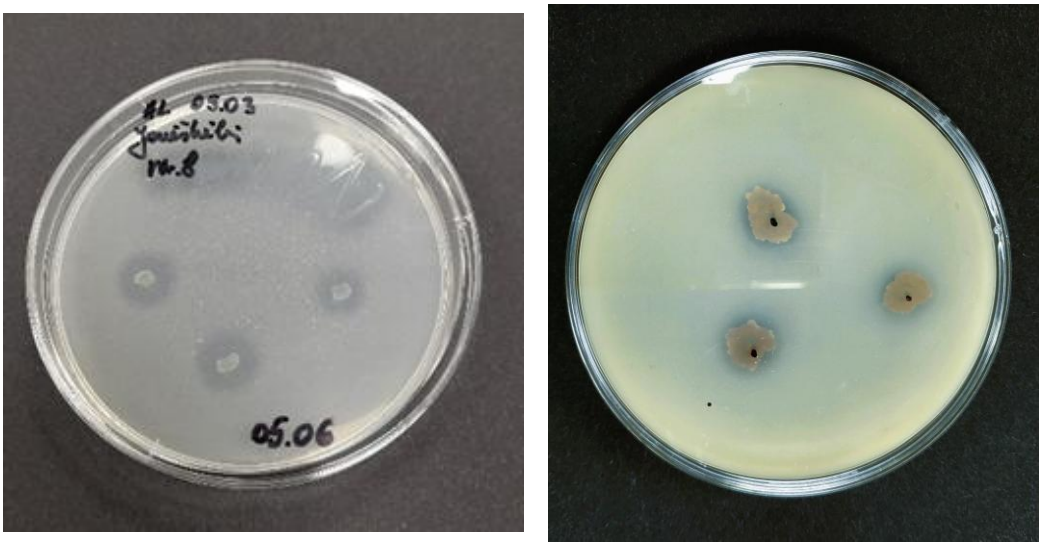
observed with growth of RUM2, RUM4, RUM10, JON3, JON4, JON6, JON8, VDU3, VDU5, VDU8, VOK2, VOK7, VEZ1, VEZ3, VEZ7, VEZ8, VEZ9 isolates.



Figure 1. Nitrogen fixation assay in semi-selective NFB medium. Isolate biomass ring formation and media colour change from green to blue showing positive reaction (left tube), control, not inoculated treatment (right tube).

Only 2 out of 50 isolates exhibited insoluble phosphate-dissolving activity, as indicated by the formation of clear zones on PVK agar plates. Isolate RUM4, formed a 9.5% higher PSI zone in the PVK agar medium than isolate JON8 (Table 2). Isolate RUM4 PSI varied and grew during each observation, increasing by 10.6% from baseline to end, and reaching 2.30 on observation day 14. The isolate JON8 PSI index was the same on day 2 and day 5 of observation, and after 7 days it rose by 0.96% and remained the same after 14 days — 2.10.

Of the 50 isolates identified, only two bacterial isolates were characterised by the dissolution of insoluble potassium on Aleksandrow's solid nutrient medium (Figure 2). The Khandeparkar's selection coefficient (KSR) for isolate JON8 was 67.3% higher than that for isolate VDU3 (Table 2). The KRS of isolate JON8 was 2.51, whilst isolate VDU3 exhibited weak solubilisation of insoluble potassium, with a KRS of only 1.50.

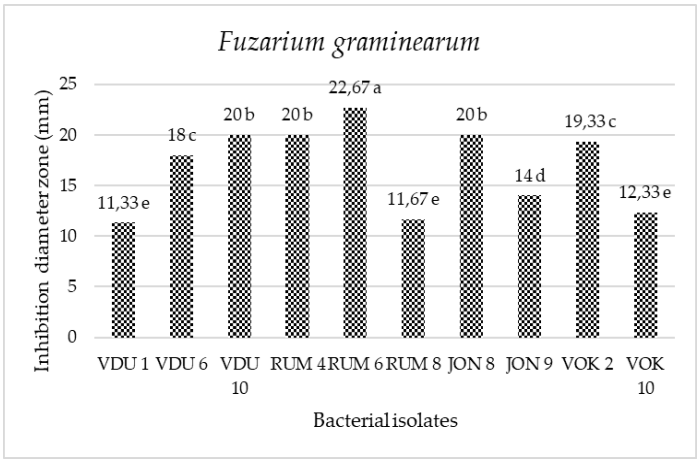


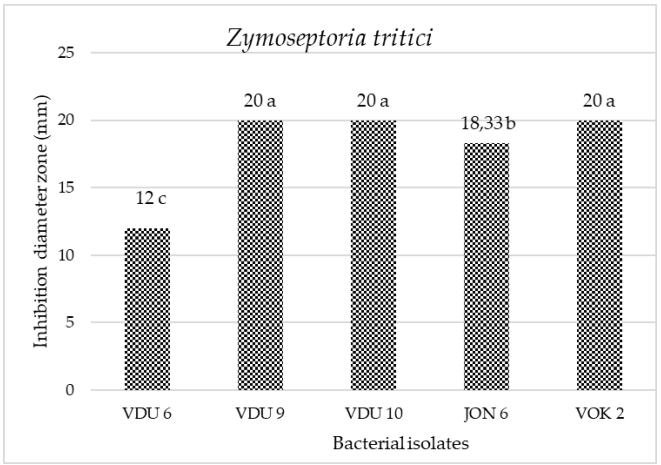
a. **b.**

Figure 2. The ability of isolate JON8 to dissolve potassium (a) and phosphorus (b), forming transparent zones around the bacteria colony.

Of the 50 isolates, only 10 bacterial isolates had antagonistic characteristics against *F. graminearum (*Table 2*).* Isolate RUM6 with a damping zone of 22.67 mm — the highest statistically significant value was observed among all the tested isolates. Isolates VDU10, RUM4, JON8, with a damping zone of—20.00 mm. Did not differ from each other but were significantly smaller than the isolate RUM6 and larger than the rest of the tested isolates. The weakest antagonistic action against the fungus *F. graminerum* were three isolates and arranged as follows; VOK10 — 12.33 mm., RUM8-11.67 mm., VDU1- 11.33 mm damping zones, although the values are different but not substantial (Figure 3a.). The antifungal activity of the VOK2 isolate against the fungus *F. graminearum* can be seen in Figure 3a.

Only five of the isolates studied exhibited antagonistic properties against the pathogenic fungus *Z. tritici* (Table 2); the biological activity observed varied among the isolates. Isolates VDU9, VDU10 and VOK2 (Figure 4b.) achieved a 20.00 mm damping zone with the highest antagonistic effect. Their values were identical and did not differ statistically from one another but were significantly higher than the activity of the other isolates. Statistically, the weakest inhibitory efficiency was achieved by isolate VDU6, whose damping zone was 12.00 mm., significantly lagging all other tested variants.





(**a**) (**b**)

Figure 3. Diameter of inhibition zone for different bacteria isolates (mm) before *F. graminearum* (a) and *Z. tritici* (b).

Note: Differences between the averages of treatments marked with different letters (a, b, c, d, e) are significant (P<0,05).



a. **b..**

Figure 4. Isolate VOK2 antagonistic activity against *Fusarium graminearum* (a) and isolate VDU9 antagonistic activity against *Zymoseptoria tritici* (b).

The Gram stain and endospore detection method showed that 24 isolates are Gram-positive and capable of forming endospores, whilst the remaining 26 isolates are Gram-negative and unable to produce endospores. Six isolates from RUM and six isolates from VDU were Gram-positive and formed endospores; five isolates from VOK, four isolates from JON and three isolates from VEZ were also Gram-positive and formed endospores. The remaining isolates were Gram-negative and unable to form spores: 7 isolates of VEZ, 6 isolates of JON, 5 isolates of VOK, 4 isolates of VDU and 4 isolates of RUM (Table 2).

After checking the ability of isolated bacteria to fix nitrogen, to dissolve phosphorus and potassium compounds inaccessible to the plant, RUM4 and JON8 isolates showed the best results. Following an assessment of their antagonistic effects against the pathogens *F. graminearum* and *Z. tritici*, two isolates, VDU10 and VOK2, were selected on the basis of the results obtained. Bacterial isolates that showed the best results were identified using a 16S rDNA gene analysis.

3.2 Phylogenetic Analysis of 16s rRNR Gene Sequences

Phylogenetic analysis based on 16S rRNA sequences showed that the RUM4 and JON8 isolates belong to the genus *Pseudomonas spp.,* the VDU10 isolate to the genus *Paenibacillus spp.,* and the VOK2 isolate to the genus *Bacillus spp.* The 16S rRNA sequences of the isolates were compared with the 16S rRNA gene sequences of representative strains. Phylogenetic trees were constructed based on the results of the analysis of the 16S rRNA gene sequences obtained.

The phylogenetic tree (Fig. 5) showed that both isolates, RUM4 and JON8, are closely related to different species of *Pseudomonas*. Isolate RUM4 is closely related to *P. picicola* P50 LR797558, with which it shares 99.84% of identity, and to *P. brassicacearum subsp. neoaurantiaca* CIP 109457 EU391388, with which it shares 99.78% of identity, as well as *P. alvandae* SWRI17 CP077080, with which it shares 99.72% of identity. The isolate JON8 is homologous to *P. kilonensis* 520-20 AJ292426, with which it shares 99.79% of identity, as well as *P. zarinae* SWR1108 CP077086 — 99.71% and *P. corugata* ATCC 29736 D84012 — 99.57%.

Other phylogenetic trees formed from bacterial isolates showed that bacterial isolate VOK2 was homologous to *Bacillus mycoides* DSM 2048 ACMU01000002 — 99.92% (Figure 6), and bacterial isolate VDU10 was homologous to *Peanibacillus tundrae* A10b EU558284 — 99.00% (Figure 7).

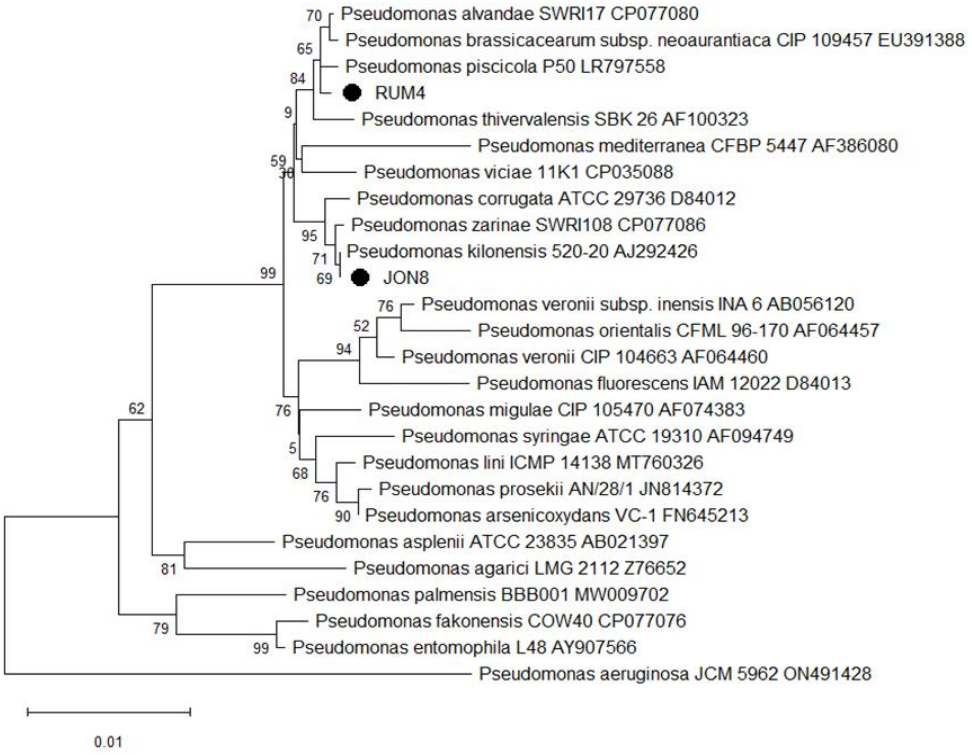


Figure 5. The phylogenetic relationships of *Pseudomonas* sp. RUM4 and *Pseudomonas* sp. JON8 within the genus *Pseudomonas* spp. were investigated using 16S rRNA gene sequence analysis. The phylogenetic tree was constructed using the MEGA 5.0 software package, employing the neighbour-joining statistical method with 1,000 bootstrap repetitions. The scale bar indicates a 0.01 change in each nucleotide position.

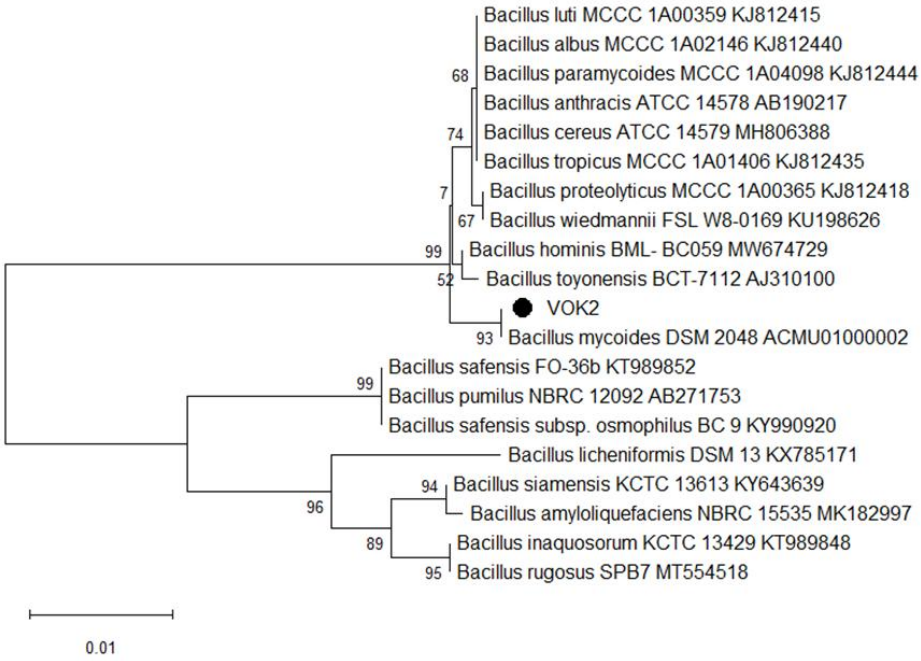


Figure 6. The phylogenetic relationships of *Bacillus* sp. VOK2 within the genus *Bacillus* spp., investigated using 16S rRNA gene sequence analysis. The phylogenetic tree was constructed using the MEGA 5.0 software package, employing the neighbour-joining statistical method with 1,000 bootstrap repetitions. The scale bar indicates a 0.01 change in each nucleotide position.

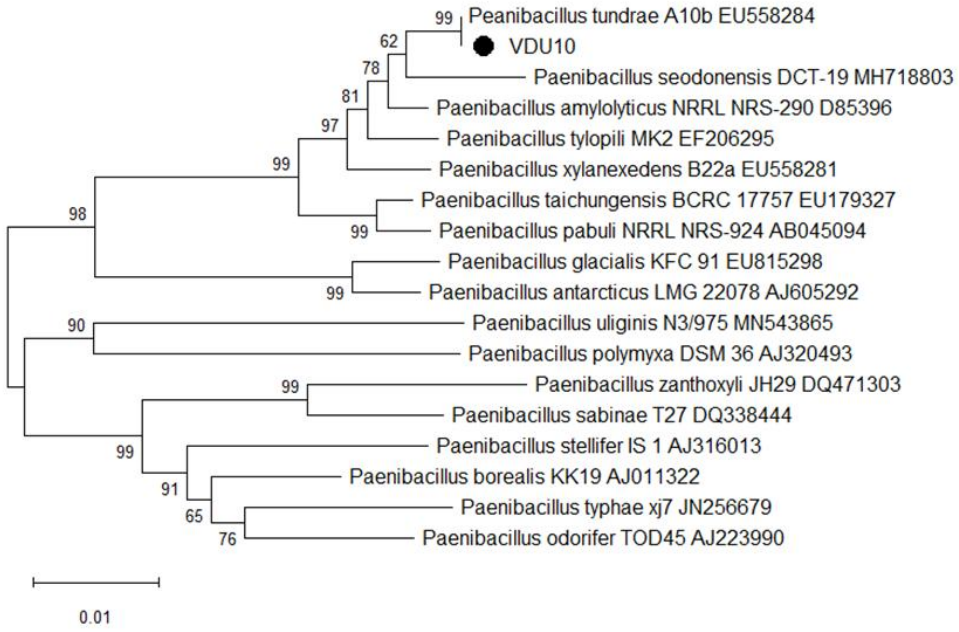


Figure 7. The phylogenetic relationships of *Peanibacillus* sp. VDU10 within the genus *Peanicillus* spp., investigated using 16S rRNA gene sequence analysis. The phylogenetic tree was constructed using the MEGA 5.0 software package, employing the neighbour-joining statistical method with 1,000 bootstrap repetitions. The scale bar indicates a 0.01 change in each nucleotide position.

According to the German TRBA 466 classification, *Pseudomonas* sp. RUM4, *Pseudomonas* ps. J, *Paenibacillus* sp. VDU10 corresponds to risk group 1, *Bacillus* sp. VOK2 is assigned to risk group 2. Risk group 1 applies to work with well-known agents that do not cause diseases in healthy adults. Risk group 2 level is intended to work with agents that present moderate risks to personnel and the environment (may cause infections through cuts, mucous membranes or inhalation of aerosols).

3.3 A study of the compatibility of isolated bacteria and plant extracts

Pseudomonas sp. RUM4 and *Pseudomonas* sp. JON8 isolates capable of capturing nitrogen, phosphorus and potassium compounds inaccessible to the plant were mixed with nettle and nettle-yarrow extracts. According to the manufacturer J3C Agri, nettle extract promotes the development of a strong root system and helps plants to absorb nitrogen and micronutrients (iron, magnesium) from the soil more easily and effectively [50]. Another mixing was carried out with nettle-yarrow extract from the Schacht manufacturer, the product description states that nettle particularly promotes plant growth, and yarrow increases plant resistance to pests and diseases [51].

Bacterial isolates were mixed with plant extracts; plating was carried out immediately after mixing, after 1 and 3 hours, after 1 and 2 weeks, and after 1 month. Immediately after mixing, *Pseudomonas* sp. JON8 (3.50×10^6 CFU ml^{-1}) remained stable and unchanged, *Pseudomonas* sp. RUM4 (ranging from 4.70×10^6 to 4.10×10^6 CFU ml^{-1}) maintained a concentration almost identical to that of the control (Figure 8). This proves that nettle extract in the short term did not significantly affect the stability of the cells of the bacteria studied. Three hours after mixing, a steady but slow decline in the number of bacterial cells is observed, P*seudomonas* sp. RUM4 from $4.1x10^6$ to $3.2x10^6$ CFU ml^{-1}, *Pseudomonas* sp. JON8 from $3.5x10^6$ to $3.2x10^6$ CFU ml^{-1}, isolate mixture *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 from $2.1x10^6$ to $1.8x10^6$ CFU ml^{-1}. From the very first week, a clear decline in the number of all isolated bacteria begins. 2 weeks later *Pseudomonas* sp. RUM4 and *Pseudomonas* sp. JON8 maintained an identical concentration of $2.00x10^6$ CFU ml^{-1} and *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 concentration drops to $8.00x10^5$ CFU ml^{-1}. After 1 month, there was an even greater reduction in the number of micro-organisms in all groups, *Pseudomonas* sp. RUM4 being the most resistant: after 1 month it maintained maximum viability ($3.50x10^5$ CFU ml^{-1}). This strain tolerated the nettle extract medium best. *Pseudomonas* sp. JON8 being

the most sensitive, its concentration dropped the most — down to $9.00x10^4$ CFU ml^{-1}. This variety is more sensitive to long-term storage in this mixture. Mixture *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 maintained a good number of live bacteria $2.20x10^5$ CFU ml^{-1} after one month. This indicates that *Pseudomonas* sp. RUM4 helps stabilise the overall viability of the mixture.

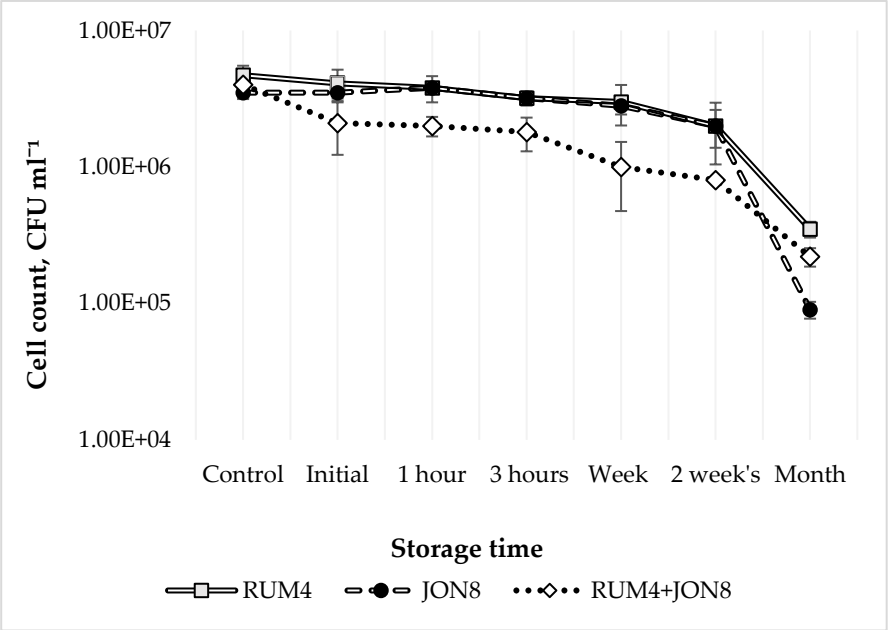


Figure 8. Compatibility of the *Pseudomonas* sp. RUM4 and *Pseudomonas* sp. JON8 isolates with nettle extract. **Note:** RUM4 — *Pseudomonas* sp. RUM4, JON8—*Pseudomonas* sp. JON8, RUM4+JON8 —*Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8.

Pseudomonas sp. RUM4 when mixed with nettle and yarrow extract, the bacterial count fell slightly immediately after mixing to $4.50x\ 10^6$ CFU ml^{-1} compared with the control ($4{,}70x\ 10^6$ CFU ml^{-1}) (Fig.8). *Pseudomonas* sp. JON8 and a mixture of isolates comprising *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8 showed an identical initial adaptation point of 3.20×10^6 CFU ml^{-1} immediately after mixing. This indicates that nettle and yarrow extract does not have a strong toxic effect on these PGPR isolates at the initial stage. During the first three hours, all samples maintained their viability perfectly at the same exponential rate of 10^6 CFU ml^{-1}. One hour after mixing, the concentration of the mixture of *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8 (from 3.20×10^6 to 3.4×10^6 CFU ml^{-1}) and *Pseudomonas* sp. JON8 (from 3.2×10^6 to 3.6×10^6 CFU ml^{-1}) had even increased slightly compared with the initial concentration (Initial). After 3 hours, a consistent but very mild decrease in concentration was observed in all variants. *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8 consortium maintained an identical count of 2.70×10^6 CFU ml^{-1} between the first and second weeks, indicating the ideal coexistence of both strains in this plant-based medium. In monocultures *Pseudomonas* sp. RUM4 and *Pseudomonas* sp. JON8 concentrations decreased slightly, remaining in the 10^6th range. One month after mixing with nettle and yarrow extract, the *Pseudomonas* sp. JON8 isolate performed worst in this medium — its concentration fell the most, to 4.40×10^4 CFU ml^{-1}, in the mixture of *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8, the highest number of viable bacteria in the entire experiment was recorded — 6.00×10^5 CFU ml^{-1}; a synergistic effect was observed in this mixture.

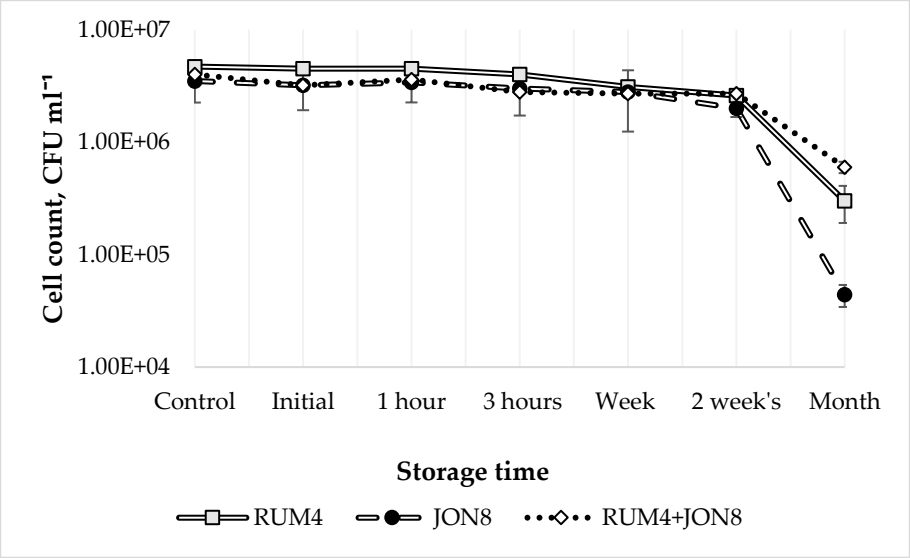


Figure 9. Compatibility of the isolates *Pseudomonas* sp. RUM4 and *Pseudomonas* sp. JON8 with nettle and yarrow extract. **Note:** RUM4 — *Pseudomonas* sp. RUM4, JON8—*Pseudomonas* sp. JON8, RUM4+JON8 — *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8.

Following testing of their fungistatic activity against plant pathogens, the two isolates *Bacillus sp.* VOK2 and *Peanibacillus* sp. VDU10 showed the best results; they were mixed with extracts of field horsetail and willow bark. J3C Agri highlights that field horsetail extract offers protection against fungal diseases; the high natural silicon (silicon dioxide) content in field horsetail mechanically strengthens plant cell walls. As a result, it becomes much more difficult for the spores of fungal diseases (e.g. powdery mildew, downy mildew, leaf spot, rust, fusarium wilt) to penetrate the plant. The manufacturer, J3C Agri, describes willow bark extract as a natural fungicide and plant immunostimulant. It is rich in salicylic acid, which acts as a "danger signal" for the plant, pre-activating the plant's immune system and triggering the production of protective substances even before the actual attack by pathogens. It promotes the growth of the root system in young plants and stimulates biological and microbiological activity in the soil [50].

Peanibacillus sp. VDU10 isolate exhibited a very high initial concentration of 2.80×10^7 CFU ml^{-1}, which was significantly higher than that of *Bacillus* sp. VOK2 (2.80×10^6 CFU ml^{-1}) and *Bacillus* sp. VOK2 +*Peanibacillus* sp. VDU10 (5.10×10^6 CFU ml^{-1}) (Figure 9). Immediately after mixing with field horsetail extract, the isolates of *Bacillus* sp. VOK2, *Peanibacillus* sp. VDU10 and *Bacillus* sp. VOK2 + *Peanibacillus* sp. VDU10 remained stable. The concentration of *Peanibacillus* sp. VDU10 increased even slightly (from $2.80\text{x}10^7$ to $3.0\text{x}10^7$ CFU ml^{-1}), whilst that of *Bacillus* sp. VOK2 (from 2.10×10^6 to 1.4×10^6 CFU ml^{-1}) and that of the mixture of *Bacillus* sp. VOK2 +*Peanibacillus* sp. VDU10 (from 5.10×10^6 to 4.4×10^6 CFU ml^{-1}) decreased slightly. This demonstrates that fresh field horsetail extract has no acute toxic effect on these strains. In the first 3 hours, all three samples showed excellent vitality. The figures fluctuated only slightly, indicating that the bacteria had successfully adapted to the pH of the extract and the biologically active substances it contained. The concentration of the mixture of *Bacillus* sp. VOK2 +*Peanibacillus* sp. VDU10 rose slightly after 3 hours to 4.90×10^6 CFU ml^{-1}, compared with the initial concentration of 4.40×10^6 CFU ml^{-1}. After one to two weeks, all the samples lost their stability. *Bacillus* sp. VOK2 was hardest hit — its concentration fell to its lowest level, 7.20×10^5 CFU ml^{-1}. *Peanibacillus* sp. VDU10 and the mixture of *Bacillus* sp. VOK2 +*Peanibacillus* sp. VDU10 decreased by one logarithmic order (from 10^7 to 10^6) but maintained a similar concentration (~ 2.10×10^6 — 2.20×10^6 CFU ml^{-1}). This indicates that it was the more resistant *Peanibacillus* sp. VDU10 strain that maintained the viability of the mixture at this stage.

Although *Peanibacillus* sp. VDU10 constituted the largest population (10^7) in the control and in the mixtures after 1 and 3 hours, its concentration began to fall after 1 and 2 weeks and had decreased to 4.30×10^5 CFU ml^{-1} after one month. This indicates that this strain is sensitive to the long-term effects of components in field horsetail extract (such as saponins or silicon compounds). *Bacillus* sp. VOK2 isolate turned out to be the most resistant in this medium. Its initial population was lower ($2.1\text{x}10^6$ CFU ml^{-1}) but maintained a high level of $3.80\text{x}10^5$ CFU ml^{-1} after 1 month. After one month, the mixture of *Bacillus* sp. VOK2 +*Peanibacillus* sp. VDU10 contained 3.40×10^5 CFU ml^{-1} of live bacteria.

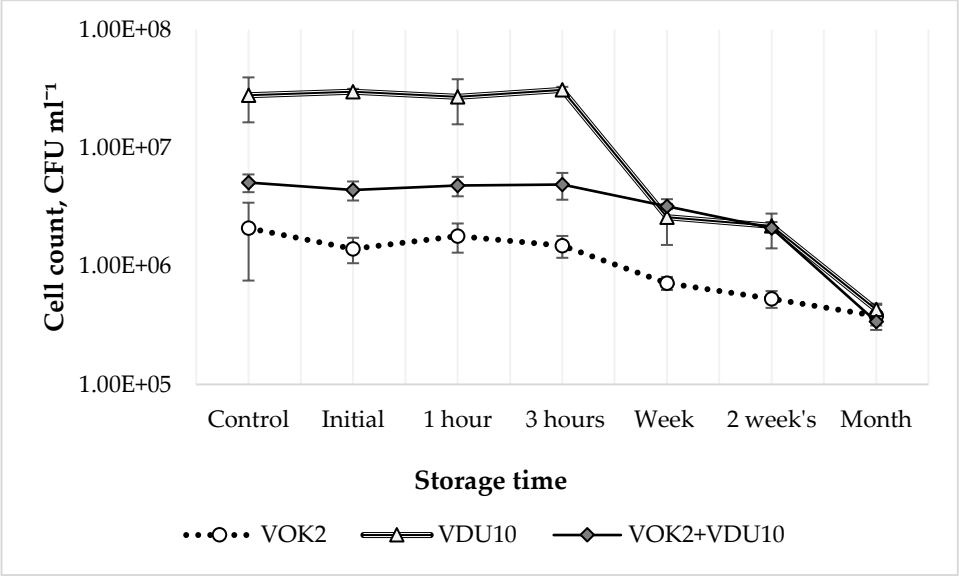


Figure 10. Isolates of *Bacillus* sp. VOK2 and *Peanibacillus* sp. VDU10 compatibility with field horsetail extract. **Note**: VOK2 — *Bacillus* sp. VOK2, VDU10 — *Peanibacillus* sp. VDU10, VOK2+VDU10 — *Bacillus* sp. VOK2+*Peanibacillus* sp. VDU10.

When the bacterial isolates were mixed with willow bark extract immediately after mixing (Initial), the bacterial concentrations remained stable. The concentration of *Bacillus* sp. VOK2 rose slightly at the start, from 2.10×10^6 to 2.40×10^6 CFU ml^{-1}. *Peanibacillus* sp. VDU10 remained relatively stable at 2.60×10^7 CFU ml^{-1} compared to control at 2.90×10^7 CFU ml^{-1}. The mixture of *Bacillus* sp. VOK2+ *Peanibacillus* sp. VDU10 also maintained a high initial viability of 4.90×10^6 CFU ml^{-1}. This indicates that willow bark extract has no toxic effect on these strains. *Peanibacillus* sp. VDU10 isolate showed exceptional resistance in the short term; after 3 hours, its concentration (2.70×10^7 CFU ml^{-1}) had almost returned to its initial level (Initial) (2.60×10^7 CFU ml^{-1}), indicating complete adaptation. The concentration of *Bacillus* sp. VOK2 had fallen by approximately half after 3 hours compared with the control (from 2.10×10^6 to 1.20×10^6 CFU ml^{-1}). This indicates temporary stress of bacteria in adapting to the acidity of the medium or phenolic compounds. After 1 to 2 weeks, the extract has a strong inhibitory effect on all variants, but *Peanibacillus* sp. VDU10 successfully maintains stability within the range of 10^7 to 10^6, demonstrating excellent resistance to tannins.

Peanibacillus sp. VDU10 isolate maintained the maximum number of live bacteria at 2.30×10^6 CFU ml^{-1} after 1 month. After one month, the mixture of *Bacillus* sp. VOK2+ *Peanibacillus* sp. VDU10 contained 5.80×10^5 CFU $ml^{-1}$of live bacteria. This is almost three times higher than the figure for *Bacillus* sp. VOK2, which is 2.00×10^5 CFU ml^{-1}. This indicates that inclusion in the consortium helps to protect the more sensitive *Bacillus* sp. VOK2 strain from complete loss during long-term storage.

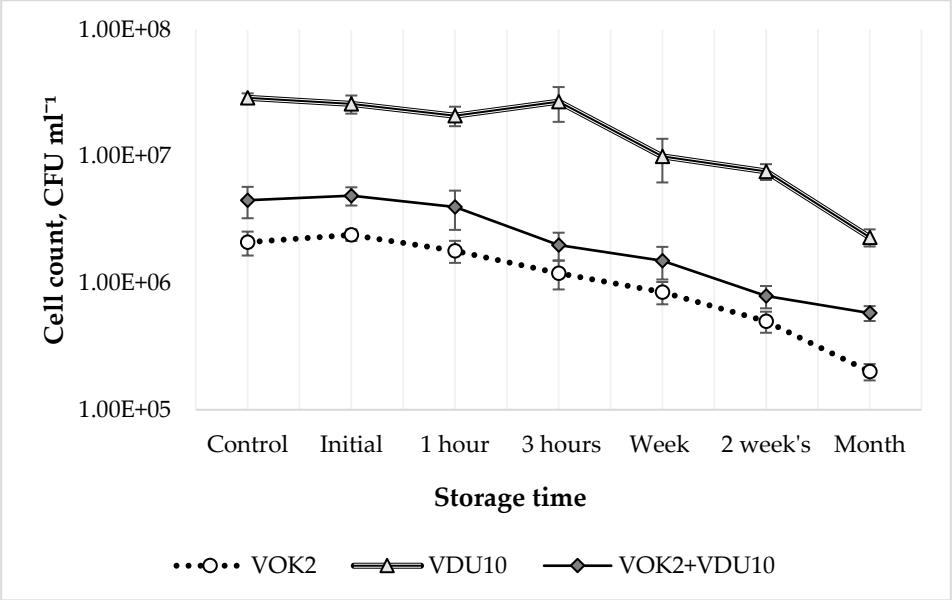


Figure 11. Isolates of *Bacillus* sp. VOK2 and *Peanibacillus* sp. VDU10 compatibility with field horsetail extract. **Note:** VOK2 — *Bacillus* sp. VOK2, VDU10 — *Peanibacillus* sp. VDU10, VOK2+VDU10 — *Bacillus* sp. VOK2+ *Peanibacillus* sp. VDU10.

3.4 *The Effect of Isolated Bacteria and Plant Extracts on the Germination and Biometric Parameters of Winter Wheat*

Winter wheat seeds have been coated with a mixture of various bacterial suspensions and plant extracts and separately according to the scheme described in the methodology (Table 1). The germination energy did not differ significantly from one biostimulant to another, although all variants were larger than the control variant and were arranged as follows: *Pseudomonas* sp. RUM4 and nettle+yarrow extract 3.5%, *Pseudomonas* sp. JON8 and *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8+nettle extract 1%, *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8 and *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract and nettle extract 0.5% each, increased the germination energy compared to control (Figure 12).

Although the germination rate for all variants was higher than that of control, the use of biostimulants *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 and *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extracts statistically significantly increased germination, which was 9.0% and 9.1% higher, respectively, than control. Other biostimulants also promoted germination: nettle+yarrow extract—7.0%, *Pseudomonas* sp. RUM4—6.5%, *Pseudomonas sp.* RUM4+ *Pseudomonas* sp. JON8+nettle extract — 5.5%, *Pseudomonas* sp. JON8 — 5%, nettle extract — 1%, but this increase in germination was small enough to be considered statistically reliable (Figure 12).

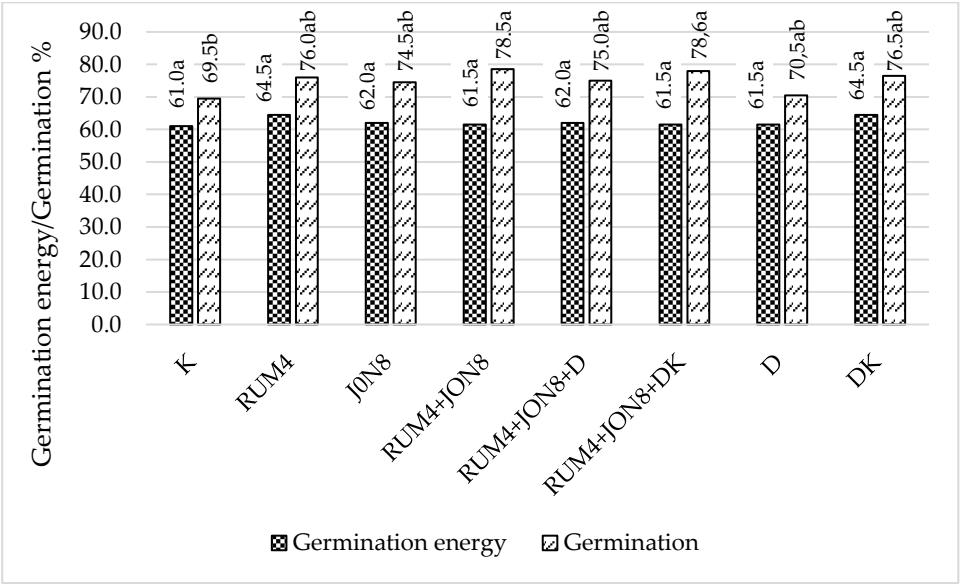


Figure 12. Winter wheat germination energy and germination using different biostimulants. **Note:** Differences between the averages of treatments marketing with different letters (a, b, c, d, e) are significant (P<0.05), K — control, biostimulants not used, RUM4 — *Pseudomonas* sp. RUM4, JON8 — *Pseudomonas* sp. JON8, RUM4+JON8 — *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8, RUM4+JON8+D — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 +nettle extracts, RUM4+JON8+DK — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 +nettle+yarrow extract, D — nettle extract, DK — nettle+yarrow extract.

The results of the study into the root length of winter wheat showed that the longest roots were produced by winter wheat treated with a mixture of: *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract—the length of the roots reached 39.18 mm, and *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 the length of the roots reached—39.13 mm (Figure 13). Compared to the control variant, these biostimulants significantly influenced the length of the winter wheat roots, respectively: *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8+nettle+yarrow extract —7.3% and *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8—7.2%. The other biostimulants used in the study had no significant effect on root length, although they did increase root length slightly compared with the control and were ranked as follows: *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract — 5.0%, *Pseudomonas* sp. JON8 -1.5%, *Pseudomonas* sp. RUM4-0.9%, nettle+yarrow extract — 0.5%, nettle extract — 0.4%.

The use of different biostimulants had no significant effect on seedling length. When winter wheat seeds were inoculated with different biostimulants, they tended to increase seedling heigh; following inoculation with *Pseudomonas* sp. RUM4 and *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 the seedlings were 11.1% taller — *Pseudomonas sp.* JON8—8.0%, *Pseudomonas sp.* RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract — 7.1%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract — 6.3%, nettle extract — 5.4%, compared to the control treatment. Only when the seeds were inoculated with a nettle and yarrow extract were the seedlings 1.4% shorter than those in the control treatment (Figure 13).

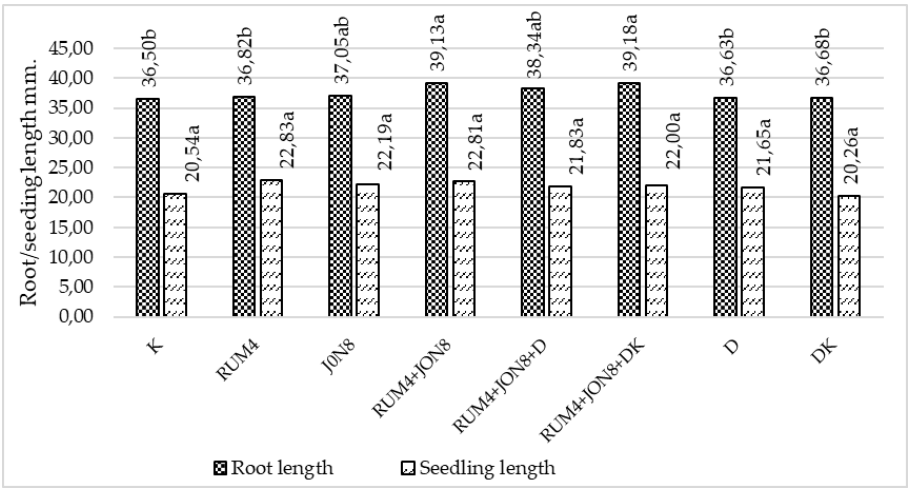


Figure 13. The effect of different biostimulants on frost damage to winter wheat and seedling length. **Note:** Differences between the averages of treatments marked with different letters (a, b, c, d, e) are significant (P<0.05), K – control, biostimulants not used, RUM4 — *Pseudomonas* sp. RUM4, JON8 — *Pseudomonas* sp. JON8, RUM4+JON8 — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8, RUM4+JON8+D — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract, RUM4+JON8+DK — *Pseudomonas* sp. RUM4+*Pseudomonas sp.* JON8+nettle+yarrow extract, D — nettle extract, DK — nettle+yarrow extract.

Winter wheat root area was statistically reliable using bacterial isolate *Pseudomonas* sp. JON8 and reached 21.13 mm^2, which was 30.9% higher than control (Figure 14). Other biostimulants also increased root area, *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8 — 13.13%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract —12.4%, *Pseudomonas* sp. RUM4 — 11.9%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract — 11.9%, nettle+yarrow extract — 7.43%, but not substantially, compared to the control (K) treatment. Only after using nettle extract, the root area was 4.3% smaller than control (K).

The total root length in all variants where different biostimulants were used was higher but not substantial. Biostimulants increased the total length of the roots and ranked as follows: *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 — 17.1%, nettle+yarrow extract (DK) — 16.3%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract — 12.4%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract—8.3%, *Pseudomonas* sp. RUM4-7.1%, nettle extract — 1.8%, compared to the control treatment (Figure 14).

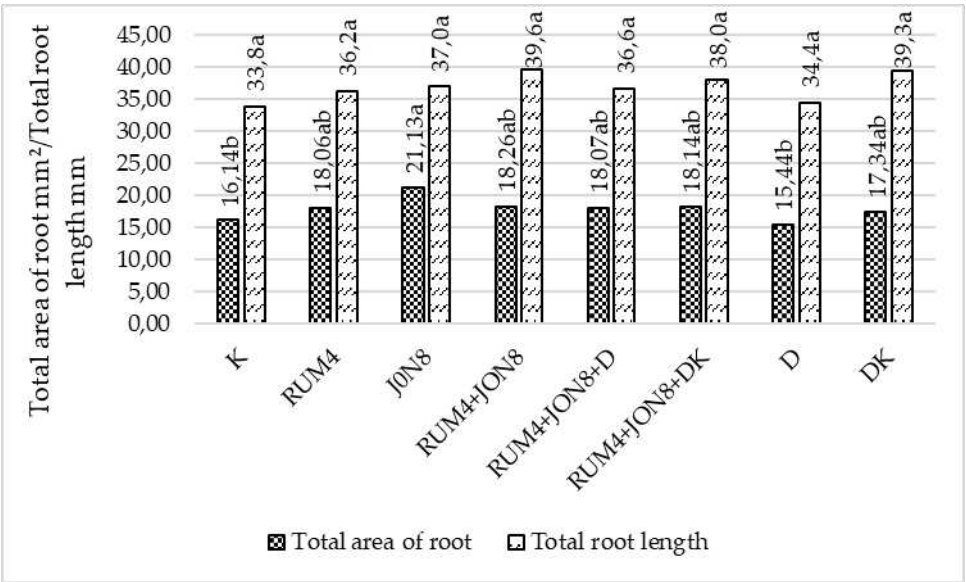


Figure 14. The area and length of winter wheat roots following the application of different biostimulants. **Note:** Differences between the averages of treatments marked with different letters (a, b, c, d, e) are significant (P<0.05), K — control, biostimulants not used, RUM4 — *Pseudomonas* sp. RUM4, JON8 — *Pseudomonas* sp. JON8, RUM4+JON8 — *Pseudomonas* sp. RUM4+ *Pseudomonas* sp. JON8, RUM4+JON8+D — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract, RUM4+JON8+DK — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract, D — nettle extract, DK — nettle+yarrow extract.

The biomass of winter wheat roots was statistically significant in two treatments, following the application of the following biostimulants: *Pseudomonas* sp. JON8 and nettle + yarrow extract, 60.0% and 55.0% higher respectively than the control variant (Figure 15). Three more biostimulants increased root biomass compared to control, *Pseudomonas* sp. RUM4 — 30.0%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract—25.0% and *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract — 12.5%, but not significantly. Following inoculation of winter wheat seeds with *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 and nettle extract the root biomass was the same as in the control treatment, amounting to 0.008 g (Figure. 15).

The green biomass of the stems was significantly highest when three biostimulants were used; these increased the green mass of the stems and were ranked as follows: nettle + yarrow extract — 33.3%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 and *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract at 20.8% each, compared with the control treatment. Biostimulants *Pseudomonas* sp. JON8, *Pseudomonas sp.* RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extracts 11.7% and *Pseudomonas* sp. RUM4 — 8.3%, increased the green mass of the stems, but not significantly compared to control. In nettle extract, the green biomass of the inoculated stem of the seeds was the same (0.0096 g) as in variant where biostimulants were not used (Figure 15).

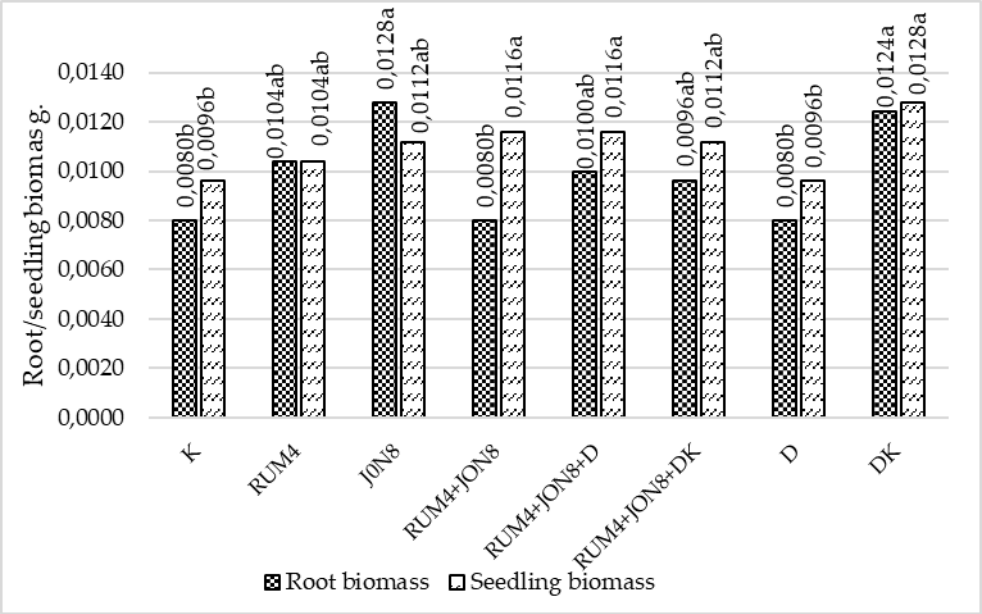


Figure 15. Winter wheat root and seedlings biomass using different biostimulants. **Note:** Differences between the averages of treatments marked with different letters (a, b, c, d, e) are significant (P<0.05), K — control, biostimulants not used , RUM4 — *Pseudomonas* sp. RUM4, JON8 — *Pseudomonas* sp. JON8, RUM4+JON8 — *Pseudomonas* sp. RUM4+*Pseudomonas sp.* JON8, RUM4+JON8+D — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract, RUM4+JON8+DK — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract, D — nettle extract, DK — nettle+yarrow extract.

The dry matter content in the roots was statistically significantly higher with the use of three biostimulants: *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 — 9.4%, *Pseudomonas* sp. JON8 and nettle extract 6.4% each, — resulted in higher dry matter accumulation in the roots compared with the control. Other biostimulants used increased the dry matter content, respectively: *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract — 4.7%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract — 4.4%, *Pseudomonas* sp. RUM4 – 1.7%, the increase was insubstantial compared to control. Following the application of the nettle and yarrow extract, the dry matter content in the roots was the lowest of all the biostimulants used, at 19.4 per cent, which was close to that of the control treatment at 19.1 per cent (Figure 16).

The highest percentage of dry matter in the stems was essentially using nettle extract and nettle + yarrow extract, and these extracts increased the dry matter content of the stems by 2.0% and — 1.2% respectively, compared to control. Other biostimulants also increased the dry matter content of the stems: *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extract—0.9%, *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract — 0.8%, *Pseudomonas* sp. RUM4—0.6% and *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8—0.5% but not significantly compared to variant where biostimulants were not used. After use of isolate *Pseudomonas* sp. JON8 isolate was applied, the dry matter content in the stems was 0.02% lower than in the control.

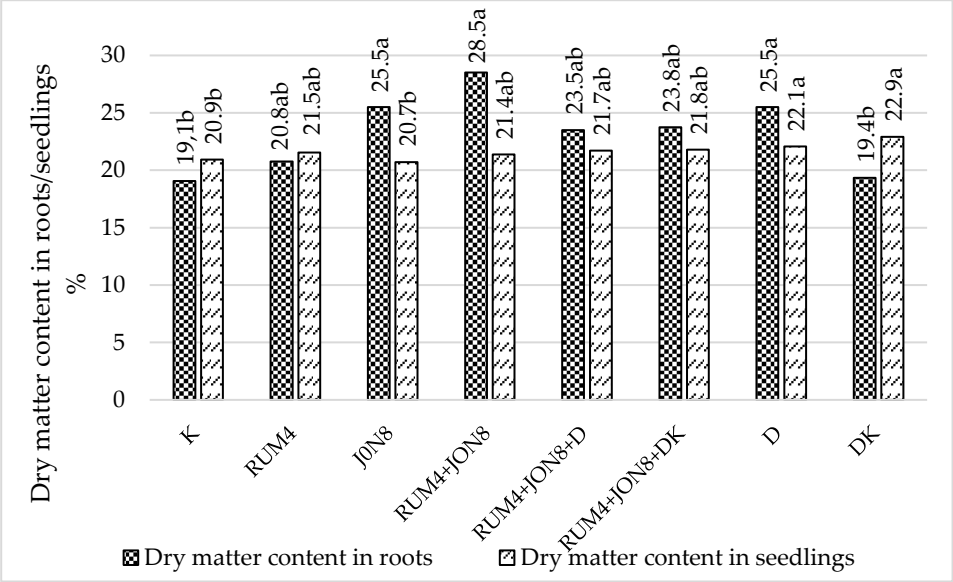


Figure 16. The dry biomass of wheat roots and seedlings, expressed as a percentage, following the application of different biostimulants. **Note:** Differences between the averages of treatments marked with different letters (a, b, c…) are significant (P<0.05), K — control, biostimulants not used, RUM4 — *Pseudomonas* sp. RUM4, JON8—*Pseudomonas* sp. JON8, RUM4+JON8 — *Pseudomonas* sp. RUM4+*Pseudomonas sp.* JON8, RUM4+JON8+D – *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle extract, RUM4+JON8+DK — *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8 +nettle+yarrow extract, D — nettle extract, DK — nettle+yarrow extract.

3. Discussion

Out of the 50 isolated bacteria, two isolates were selected: JON8, which was characterised by its ability to fix nitrogen and solubilise insoluble phosphorus and potassium compounds, and RUM4, which could fix nitrogen and solubilising insoluble phosphorus compounds. Thus, the two isolates have the highest biological activity against *Z. tritici* and *F. graminearum.* Phylogenetic analysis revealed that JON8 was most closely related to *P. kilonensis*, RUM4 to *P. piscicola*, VDU10 to *P. tundrea*, and VOK2 to *B. mycoides*. According to data presented in the scientific literature, *Pseudomonas* is often predominant amongst the bacteria in the plant rhizosphere and influences plant growth by improving the availability of soil nutrients to plants, secreting growth-promoting hormones, suppressing soil-borne pathogens, and producing antibiotics, siderophores and lytic enzymes [52]. In the rhizosphere, some species of *Pseudomonas* act as plant growth-promoting rhizobacteria (PGPR), improving nutrient uptake through mechanisms such as nitrogen fixation [53]. Both isolates under investigation, which are most closely related to the *P. kilonensis* JON8 and *P. piscicola* RUM4 strains, demonstrated growth in the nitrogen-free Ashby and NFb media. But the genome data of the species *P. kilonensis* and preliminary taxonomic studies, based on the research carried out by Sikorski et al., confirm that it naturally lacks the nif gene complex responsible for nitrogenase synthesis [54]. Therefore, the growth of the *P. kilonensis* JON8 isolate in Ashby medium can be explained by its oligonitrophilic properties, which enable the bacteria to efficiently collect and utilise minimal traces of nitrogen present in the agar components of the culture medium and in the ambient air [55], whilst the colour change (alkalisation) of the NFb medium was caused not [52] by the secretion of ammonia, but by the metabolism of the carbon source (malate), which leads to an increase in the medium's pH [56]. Furthermore, it has been established that the *P. kilonensis* F113 stem indirectly contributes to nitrogen availability in the rhizosphere by stimulating biological nitrogen fixation in neighbouring microbial communities. Combes-Meynet et al. study indicates that this strain produces the secondary metabolite 2.4-diacetylphloroglucinol (DAPG). Low concentrations of DAPG act as a signalling molecule and induce the expression of nitrogen fixation in nif genes in *Azosrillum brassiense* S245 bacteria [57]. Another isolate identified in the study as most closely related to the *P. piscicola* RUM4 strain; in agriculture (in soil and plants), it is not regarded as a traditional or widely used agent. In the scientific literature, this species is mainly mentioned in the context of aquaculture (fisheries) [58, 59] and not in soil microbiology. The species was officially described relatively recently, in 2021, by Duman M. et al. [58]. It is important to note that *P. piscicola* has its origins in research on fish, so its presence in the rhizosphere often indicates the species' plasticity — its

ability to adapt to different niches; we can therefore conclude that, in nitrogen fixation, it may exhibit a similar metabolic behaviour to the *P. kilonensis* JON8 isolate. But both tested strains are newly isolated wild isolates, which increases their likelihood of diazotrophic potential. In the natural soil ecosystem, microorganisms are constantly exposed to horizontal gene transfer, therefore, strains JON8 and RUM4 were able to acquire functional nif genes from neighbouring rizospheric diazotrophs[60]. In scientific literature, it is known that some members of the genus *Pseudomonas* can carry out this process. Certain specific diazotrophic strains are characterised by their ability to fix atmospheric nitrogen. These micro-organisms include the *Pseudomonas stutzeri* strains A1501 and DSM4166 [61]. *Pseudomonas azotifigens* 6HT33b^T and the *Pseudomonas sp*. strain K1 also possess this property [62].

The study assessed the ability of the isolated bacteria to dissolve insoluble phosphorus compounds. The most important element of the phosphorus solubilisation mechanism is the production of gluconic acid from glucose. This reaction is very important for the solubilisation of phosphates, as gluconic acid lowers the pH and chelates the cations bound to the phosphate, thereby increasing its availability to plants [63]. A study conducted by Safari M. et al., examining the application of nanocarriers in agriculture, found that the strain *Pseudomonas kilonensis* PK11 exhibited exceptional stability and outperformed the other strains studied in solubilising insoluble phosphorus sources, such as tricalcium phosphate and hydroxyapatite [64]. Our study also confirmed the results of this study that the isolate, most closely related to *P. kilonensis* JON8, can dissolve insoluble compounds of phosphorus. The study identified *Pseudomonas spp.* isolates RUM4 and JON8, which had phosphorus solubilisation indices (PSI) of 2.3 and 2.1 respectively, and which effectively solubilised insoluble phosphates; these results are consistent with those reported by Ibietiela S. et al. study, where the authors found that the isolated strain of *Pseudomonas xiamenensis* had a high 3.15 phosphorus dissolution index (PSI) [65].

In assessing the activity of potassium-solubilising bacteria (KSB) isolated from the rhizosphere in solid Aleksandrow medium, the data from our study confirm the pattern described in the literature, namely that isolates of the genus *Pseudomonas* exhibit a marked functional potential to mobilise insoluble minerals. Research by other scientists also confirms the ability of the isolate we identified, closely related to *P. kilonensis* JON8, to dissolve potassium. In a study conducted by Jabin P. and Ismail S., it was found that the *Pseudomonas sp*. (KSB-PD-1-A) strain produced a maximum potassium solubilisation index (KSI = 3.57), determined by a zone of transparency 3.00 cm in diameter surrounding a 1.17 cm colony [66]. Although the zone in the solid medium indicates initial activity, the study by Zhao et al., using *Pseudomonas aeruginosa* A10, reveals that this strain released 21.78 ppm of potassium in liquid medium, actively secreting lactic, citric, acetic and succinic acids [67]. The strain we isolated, which is closely related to *P. kilonensis* JON8, achieved a Khandeparkar selection ratio (KSR) of 2.51 in solid medium, which demonstrates that the clear zone exceeded the diameter of the colony itself by more than 2.5; the results obtained indicate reliable potassium-dissolving activity of this isolate, although in the studies by Herdiyantoro et al. on maize rhizosphere isolates, the most effective strains were characterised by an exceptionally high Khandeparkar selection coefficient (KSR ranging from 4.00 to 5.97), which correlated strongly with the ability to acidify the environment (pH ranges of 3.28—3.64) due to the intensive secretion of organic acids [38]. According to the KSR indicator *P. kilonensis* JON8 can be classified into a group of viable, plant-growth-promoting rhizobacteria (PGPR).

F. graminearum (Fusarium head blight) is a fungal disease prevalent worldwide; the isolates identified in our study, which were most closely related to *B. mycoides* VOK2 and *P.* VDU10, exhibited broad-spectrum antifungal activity against *F. graminearum*. It is known that species of the genus *Bacillus* inhibit plant pathogens by producing lipopeptide antibiotics, which are the main factors contributing to their biocontrol efficacy [68]. The inoculant isolated in our study, which is most closely related to *B. mycoides* VOK2, was able to inhibit the growth of *F. graminearum* and confirmed the findings of other researchers. In a study by Liao Y. et al., the isolated strain *B. velezensis* FXJ exhibited a significant inhibitory effect against *F. graminearum.* Antifungal studies have shown that *B. velezensis* FXJ fermentation supernatant inhibited the growth of *F. graminearum* mycelium, suggesting that the antagonistic activity is primarily due to the antimicrobial metabolites secreted. Treatment with FXJ also caused significant morphological abnormalities in *F. graminearum* hyphae and disrupted the integrity of the cell wall or membrane [68]. Such structural damage is mainly associated with the activity of antimicrobial peptides or cell wall-breaking enzymes, which are consistent with the typical antifungal mechanisms described in the *Bacillus* strains [69]. The results of Mahmoud A. F. study, showed that *Bacillus subtilis* bacteria act as an effective means of biological control, significantly inhibiting the pathogenic fungus *F. graminearum* under laboratory conditions and in a greenhouse. In

the study, the inhibition of *F. graminearum* growth caused by *B. subtilis* strains was attributed to the production of antimicrobial compounds or competition for nutrients and space [70]. The antifungal activity shown by our isolated strain *Bacillus mycoides* VOK2 against *Z. tritici* complements and expands recent scientific insights into the application of the genus *Bacillus* to biocontrol. Allioui et al. found, that the newly isolated strains of *B. subtilis* and *B. simplex* have significant potential to inhibit the pathogen responsible for *Septoria tritici* leaf spot in wheat, which confirms the versatility of bacteria of this genus. However, the effectiveness of biocontrol agents remains specific to a particular strain [71]. This was demonstrated by a study conducted by Platel et al., which found that the *B. velezensis* S1 strain exhibited a stronger antifungal effect than the S6 strain, due to qualitative and quantitative differences in the synthesised lipopeptides (iturins, surfactins, fengicins). Since the bacterium *Bacillus* directly inhibits pathogens through extracellular metabolites [72], it can be argued that the ability of *B. mycoides* VOK2 to inhibit the growth of *Z. tritici* in vitro is determined by its secreted lipopeptides and enzymes and, according to literature, the *B. mycoides* species, unlike many other representatives of *Bacillus*, has the ability to induce systemic plant resistance (ISR) [73], therefore, strain VOK2 can provide even more complex wheat protection under outdoor conditions in the future.

The strain VDU10, which was isolated during the study and is most closely related to *P. tundrae*, demonstrated reliable in vitro antifungal activity against *F. graminearum.* These results are supported by the latest data, in which Peng et al. described the efficacy of the *Paenibacillus polymyxa* A818 strain against *F. graminearum* (with a 55 per cent inhibition of mycelial growth). *P. polymyxa* A818 not only physically inhibits the spread of the fungus, but also, at the transcriptomic level, suppresses the key genes involved in mycotoxin biosynthesis (TRI1, TRI4, TRI5, TRI8), thereby reducing the concentration of trichothecenes in the grain [74]. Given that the VDU10 isolate we identified exhibited strong (20 mm) inhibition zones, it would be worthwhile in future to assess whether *P. tundrae* VDU10 also possesses the ability to block the synthesis of *F. graminearum* toxins, which would provide additional economic value in terms of protecting grain quality. The scientific literature states that *Paenibacillus* spp. can also combat plant phytopathogens through various mechanisms: including the production of lipopeptides (e.g. fusaricidin, paenimycin and pelgipeptin) [75], the induction of systemic resistance (ISR), hydrolytic enzymes (chitinase, cellulase and glucanase) and volatile organic compounds. These properties enable *Paenibacillus* strains to inhibit the growth of fungi such as *Fusarium oxysporum, F. solani, Rhizoctonia solani, Botrytis cinerea* and *Colletotrichum gloeosporioides.* It should be noted that several strains of *Paenibacillus*, including *P. polymyxa*, *P. illinoisensis* KJA-424, *P. lentimorbus* B-30488 and *P. tegi* JCK1400, effectively control fungal diseases of plants [76]. The two isolated strains close to *B. mycoides* VOK2 and *P. tundrae* VDU10 are in line with the European Union's sustainable agricultural strategies, proposing an environmentally friendly alternative that can reduce the use of chemical pesticides and inhibit the development of pathogen resistance. According to comparative studies of the genus *Paenibacillus*, VDU10 isolates have a perspective not only to inhibit the growth of *F. graminearum*, but also to genetically block the biosynthesis of trichothecenes, thereby directly improving the quality and safety of grain production.

Our research provides new insights into the compatibility between PGPR and plant extracts, an area that has been little studied to date. Most scientific studies focus solely on bacteria or plant extracts; however, there is a lack of research into their compatibility and their effect on cell viability. Our study revealed that all the isolates examined, which were closely related to *P. picicola* RUM4, *P. kilonensis* JON8, *B. mycoides* VOK2 and *P. tundrea* VDU10, were compatible with biologically active plant extracts (nettle, nettle and yarrow, field horsetail, and willow bark). At baseline (Initial) and within the first 3 hours, the bacterial concentration remained stable within 10^6—10^7 CFU ml^{-1}, demonstrating that the plant extracts did not have bactericidal effects on the tested PGPR. After one month, the instability of the *P. kilonensis* JON8 isolate became apparent; the bacterial cell count decreased significantly following mixing with both nettle extract and nettle and yarrow extract; this indicates that the components present in the extracts had an inhibitory effect on this strain over a prolonged period. In other variants, after one month's incubation, a decrease in bacterial cell count was also observed following mixing with nettle and nettle+yarrow extracts and *Pseudomonas spp.* isolates RUM4 and JON8, as well as with *P. kilonensis* JON8 alone; with the concentration of the mixtures falling to 10^4—10^5 CFU ml^{-1}. Both nettles (caffeic and chlorogenic acids) and yarrow (apigenin and luteolin) are rich in phenols. These compounds can disrupt the integrity of bacterial cell membranes and of destabilizing their conductivity [13, 77] which results in a decrease in bacterial cell counts. The greatest stability was observed in the combined multi-component mixture consisting of *Pseudomonas spp.* isolates RUM4 and JON8 and a nettle and yarrow extract. In this variant, a strong synergistic interaction was observed between the two bacterial strains and the plant phytocomponents. Thanks to this synergy, it was possible to neutralise the negative long-term effects of the extracts

previously experienced by the single JON8 isolate; this phenomenon is linked to the dual dynamics of the interaction between phytochemical components and microorganisms: once the initial toxic effect of the plant extracts has subsided, the abundant water-soluble polysaccharides present in the nettle extract begin to function as a prebiotic carbon source, which bacteria utilise to generate energy and sustain survival under adverse conditions. Furthermore, the nitrogen, iron and calcium compounds present in nettle extract act as an additional metabolic stimulant [78].

Another combination was characterised by pronounced synergistic interaction. This stable combination consisted of isolates close to *B. mycoides* VOK2+*P. tundrea* VDU10 and willow bark extract. The compatibility of these bacteria with phytocomponents can be explained by the specific chemical composition of the extract. Willow bark is rich in phenolic glycosides, particularly salicin. Salicin naturally breaks down into salicylic acid in the environment [12]. It is well established in the scientific literature that species of the genus *Paenibacillus* are tolerant of salicylic acid compounds. These bacteria often express β-glucosidase enzymes, which enable the effective hydrolysis and breakdown of plant glycosides. The bacteria successfully metabolise the released glucose as an additional source of carbon and energy[79]. Furthermore, salicylic acid may act as a signalling molecule. It stimulates the formation of a bacterial biofilm, which reliably protects cells from the harmful effects of phytochemicals [80]. Furthermore, bacteria of the genera *Bacillus* and *Paenibacillus* have an evolutionary advantage — they form metabolically inactive endospores if the concentration of the extract is too high or if the flavonoids it contains cause temporary osmotic or oxidative stress, which allows them to survive and subsequently return to a vegetative (active) state[81]. Many strains of *Bacillus* (e.g. *B. subtilis, B. velezensis*) and *Paenibacillus* (e.g. *P. polymyxa*) have specific enzymatic pathways that allow them to break down aromatic phenolic compounds. They can use salicylic acid as an alternative source of carbon and energy [82]. After one month of incubation, a decrease in bacterial cell count was observed in all mixtures containing extracts and isolates of field horsetail and willow bark that were closely related to *B. mycoides* VOK2 and *P. tundrea* VDU1; the concentration of the mixtures fell to 10^5—10^6 CFU ml^{-1}, which is attributed to the phytochemical toxicity of the willow and field horsetail extracts. The tannins and salicylic acid derivatives present in willow bark disrupt the integrity of the cytoplasmic membrane of Gram-positive bacteria, causing the cells to disintegrate. The synergistic action of the flavonoids and phenolic acids in astragalus generates intracellular oxidative stress and inhibits key bacterial enzymatic systems, resulting in the rapid death of vegetative cells in a closed system [83, 84].

The biotransformation of the mixture in a closed system over time results in rapid depletion of nutrients and the enzymatic degradation of secondary plant metabolites (especially polyphenols). These processes eventually lead to a decrease in the number of bacterial cells, and the initial biological activity of the extracts is lost. To eliminate this negative time factor effect and ensure maximum synergistic efficiency of both components, it is recommended to pour bacterial isolates and vegetable extracts immediately before the technological process and spray immediately after mixing.

The biostimulant and biofungicidal effects of plant-growth-promoting rhizosphere bacteria (PGPR) on various agricultural crops have been extensively documented in the scientific literature. However, the purpose of this study was to assess the effectiveness of the overall use of complex mixtures consisting of PGPR and plant extracts with different mechanisms of action. Such studies are very limited, but very relevant. One of the mechanisms of action of this synergy is described by Patel at al., noting that organic biostimulants of plant origin directly activate the metabolism of bacteria of the genus *Pseudomonas*. The biological mechanism suggests that *Pseudomonas spp*. stimulate seed germination more effectively than plant extracts [85]. Bacteria synthesize phytohormones, such as gibberellins and auxins (indole-3-acetic acid) [86], which activate enzymes for the breakdown of endosperm starch (alpha-amylases) in the seed during germination [87]. Nettle extract provides a large amount of easily assimilated amino acids and organic nitrogen [88], whilst common yarrow extract enriches the environment with free sugars and structural polysaccharides; the addition of these nutrients (sources of carbon and nitrogen) to the bacterial mixture acts as a signal that encourages the bacteria to multiply more rapidly, colonise the seed more effectively with a protective biofilm [85] and protect it from environmental pathogens [89]. Furthermore, components of bacterial cell walls (lipopolysaccharides, flagellin), when interacting with plant phenolics, trigger induced systemic resistance (ISR). As a result of this effect, the plant strengthens its cell walls, develops resistance to disease and drought, and can channel all its energy into rapid growth [90]. The results of our study showed that none of the biostimulants used had a statistically significant effect on the initial germination energy of winter wheat, but they did significantly increase the final seed germination rate. The highest reliable germination rate was observed when winter wheat seeds were inoculated with the bacterial consortium *P. kilonensis* JON8 + *P. piscicola* RUM4 (78.5%) and the with mixture *P. kilonensis* JON8+*P. kilonensis* RUM4+DK (78%).

The results of our study complement the research by Iparragiurre et al., which assessed the effectiveness of extracts of the seaweed *Macrocystis pyrifera* and their combination with the plant-growth-promoting bacterium *Azospirillum brasilense* on lettuce. The use of bacteria and algae in the mixture (Az39 + AE) increased the germination energy of lettuce by 62%, and germination rate by 71% compared to the control group [91].

The effect of bacteria of the genus *Pseudomonas* on plants involves the direct stimulation of their hormonal system. Data from studies by Xu et al. show that *Pseudomonas* strains, when exposed to bioactive plant compounds, begin to synthesise indole-3-acetic acid (IAA) — the main phytohormone in the auxin class — at a higher rate [92]. The increased concentration of IAA in the rhizosphere stimulates root elongation and the development of lateral roots and root hairs, which directly results in a larger total root surface area and improves the absorption of water and nutrients [93]. The mechanisms described by the authors are also confirmed by the results of our study, in which the largest root area was formed by winter wheat seeds inoculated with a single isolate that is phylogenetically closest to the *P. kilonensis* JON8 strain. This treatment significantly increased the root area by as much as 30.9% compared to control. Such a significant change indicates that the above-mentioned isolate has an exceptional ability to promote morphogenesis of lateral roots and rhizomes, thereby increasing the total root absorption surface area. On the other hand, statistically significant maximum root lengths were observed in winter wheat, which were inoculated with a consortium of bacterial isolates *P. kilonensis* JON8 + *P. piscicola* RUM4 (39.13 mm), as well as a three-component combination in which this consortium was combined with extracts of stinging nettle and yarrow (39.18 mm). This indicates the advantage of the synergy between the microbial consortium and the plant extract over the individual components. Our findings are consistent with those of Ibrahim et al., who reported that inoculating wheat with *Pseudomonas poae* strains resulted in a significant increase in root length of up to 33% due to active IAA secretion [94].

Although the nettle and nettle+yarrow extracts did not stimulate root growth, they did increase the biomass of the wheat seedlings and the dry matter content of the stems by a statistically significant amount. This was influenced by the mineral elements (K, N, Ca, Fe), amino acids and phenolic compounds present in nettle and yarrow extracts, which act as a direct nutritional substrate; the plant directs the energy it receives not towards root growth, but towards increasing tissue density and strengthening cell walls [95]. It should be noted that all mixtures in which *Pseudomonas* bacteria were combined with plant extracts (RUM4+JON8+D and RUM4+JON8+DK) also maintained high stem biomass (0.0112—0.0116 g). These findings provide scientific evidence that, upon receiving the metabolites of the extracts, the plant directs its energy not towards cell elongation but towards tissue densification. Activated bacteria mobilise and transport the mineral elements present in the extracts to the plant cells more effectively, which results in an increase in seedling weight [95]. Our research contributes to the study by Ullah et al., which indicated that the use of 50% FC, combined with algal BC and PGPR, significantly increased the fresh and dry matter of maize shoots and roots, as well as root length, compared with the control group [96].

In view of the results obtained, it would be advisable to direct further research towards field trials under production conditions, with a view to elucidating the synergistic effects of the mixtures on the productivity and yield of winter wheat.

Conclusions

Research into biostimulants is increasingly important from both agricultural and environmental perspectives, particularly considering the withdrawal of various active substances from the market and the growing problem of pathogen resistance. In this study, two bacterial isolates with plant growth-promoting properties were selected: JON8, capable of nitrogen fixation and the solubilisation of phosphorus and potassium compounds, and RUM4, capable of nitrogen fixation and phosphorus solubilisation. Following an assessment of antagonistic activity against *Zymoseptoria tritici* and *Fusarium graminearum*, two additional bacterial isolates, VDU10 and VOK2, were selected. Sequence homology analysis showed that JON8 was most closely related to *Pseudomonas kilonensis*, RUM4 to *Pseudomonas piscicola*, VDU10 to *Pseudomonas tundrae*, and VOK2 to *Bacillus mycoides*.

The compatibility of plant growth-promoting rhizobacteria (PGPR) with plant extracts was subsequently evaluated. Two combinations exhibited synergistic interactions: *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8+nettle+yarrow extracts, and *Bacillus* sp. VOK2+*Pseudomonas* sp. VDU10+willow bark extract.

Assessment of the effects of bacterial isolates and plant extracts on winter wheat germination and biometric parameters demonstrated that the combination of RUM4 and JON8 with nettle+yarrow extracts exerted a synergistic effect on seed germination (9.1%) and root length (7.3%). In contrast, the combination of *Pseudomonas* sp. RUM4+*Pseudomonas* sp. JON8

+ nettle extract did not demonstrate a synergistic interaction. Nevertheless, both treatments showed a tendency to improve winter wheat germination and biometric parameters.

This study addresses the growing need for environmentally sustainable alternatives to synthetic pesticides and fertilisers. The findings provide new insights into biostimulant formulations combining plant growth-promoting and fungistatic properties. Further research under field conditions is needed to evaluate their efficacy, stability, and potential for practical application in sustainable agriculture.

Data Availability

The DNA/RNA sequence data generated and analyzed during this study have been deposited in the GenBank database (National Library of Medicine) and are available under the following accession numbers: PZ933729, PZ933831, PZ933857, and PZ933872.

Author contributions: A.B, Z.K designed the experiments and was involved in the visualization and writing—original draft, J.K provided experiment assistance, reviewed and edited the manuscript and were the project administrators and supervisors, S.S, Z.K and A.G reviewed and edited the manuscript and were the project administrators and supervisors. All authors have read and agreed to the published version of the manuscript.

Funding:

The reported work in this article was partially supported by the Ministry of Education, Science and Sports of the Republic of Lithuania and Research Council of Lithuania (LMTLT) under the Program 'University Excellence Initiative' Project 'Development of the Bioeconomy Research Center of Excellence' (BioTEC), agreement No S-A-UEI-23-14.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Acknowledgments

PhD studies are funded by the Lithuanian Research Council. We acknowledge JSC Bioenergy LT and CLIMAGRO LT Research Infrastructure.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. K. P. Pawłowski and G. Sołtysiak, "The Potential Impact of the European Green Deal on Farm Production in Poland," *Sustainability (Switzerland)*, vol. 16, no. 24, 2024, doi: 10.3390/su162411080.
2. M. Ricci, L. Tilbury, B. Daridon, and K. Sukalac, "General principles to justify plant biostimulant claims," 2019. doi: 10.3389/fpls.2019.00494.
3. R. Tyśkiewicz, A. Nowak, E. Ozimek, and J. Jaroszuk-ściseł, "Trichoderma: The Current Status of Its Application in Agriculture for the Biocontrol of Fungal Phytopathogens and Stimulation of Plant Growth," 2022. doi: 10.3390/ijms23042329.
4. P. Vejan, R. Abdullah, T. Khadiran, S. Ismail, and A. Nasrulhaq Boyce, "Role of plant growth promoting rhizobacteria in agricultural sustainability-A review," *Molecules*, vol. 21, no. 5, 2016, doi: 10.3390/molecules21050573.
5. M. Ruzzi and R. Aroca, "Plant growth-promoting rhizobacteria act as biostimulants in horticulture," 2015. doi: 10.1016/j.scienta.2015.08.042.
6. S. Velten, J. Leventon, N. Jager, and J. Newig, "What is sustainable agriculture? A systematic review," 2015. doi: 10.3390/su7067833.

7. P. Yang, A. Condrich, S. Scranton, C. Hebner, L. Lu, and M. A. Ali, “Utilizing Plant Growth-Promoting Rhizobacteria (PGPR) to Advance Sustainable Agriculture,” 2024. doi: 10.3390/bacteria3040030.
8. N. A. Akram *et al.*, “Phytoextracts as Crop Biostimulants and Natural Protective Agents—A Critical Review,” 2022. doi: 10.3390/su142114498.
9. P. Calvo, L. Nelson, and J. W. Kloepper, “Agricultural uses of plant biostimulants,” 2014. doi: 10.1007/s11104-014-2131-8.
10. B. Maričić *et al.*, “Non-Aerated Common Nettle (Urtica dioica L.) Extract Enhances Green Beans (Phaseolus vulgaris L.) Growth and Soil Enzyme Activity,” ., vol. 12, no. 12, p. 2145, Dec. 2022, doi: 10.3390/life12122145.
11. M. I. Dias *et al.*, “Chemical composition of wild and commercial Achillea millefolium L. and bioactivity of the methanolic extract, infusion and decoction,” *Food Chem.*, vol. 141, no. 4, 2013, doi: 10.1016/j.foodchem.2013.07.018.
12. H. Mutlu-Durak, Y. Arikan, and B. Y. Kutman, “Willow (Salix babylonica) Extracts Can Act as Biostimulants for Enhancing Salinity Tolerance of Maize Grown in Soilless Culture,” *Plants*, vol. 12, no. 4, 2023, doi: 10.3390/plants12040856.
13. M. Villalva *et al.*, “Antioxidant, Anti-Inflammatory, and Antibacterial Properties of an Achillea millefolium L. Extract and Its Fractions Obtained by Supercritical Anti-Solvent Fractionation against Helicobacter pylori,” *Antioxidants*, vol. 11, no. 10, 2022, doi: 10.3390/antiox11101849.
14. M. J. Hodson, P. J. White, A. Mead, and M. R. Broadley, “Phylogenetic variation in the silicon composition of plants,” *Ann. Bot.*, vol. 96, no. 6, 2005, doi: 10.1093/aob/mci255.
15. J. Vivancos, R. Deshmukh, C. Grégoire, W. Rémus-Borel, F. Belzile, and R. R. Bélanger, “Identification and characterization of silicon efflux transporters in horsetail (Equisetum arvense),” *J. Plant Physiol.*, vol. 200, 2016, doi: 10.1016/j.jplph.2016.06.011.
16. M. Boukhari, R. Asencio-Vicedo, M. Cerdán, A. Sánchez-Sánchez, J. D. Jordá, and B. Ferrández-Gómez, “Foliar Application of Equisetum arvense Extract Enhances Growth, Alleviates Lipid Peroxidation and Reduces Proline Accumulation in Tomato Plants Under Salt Stress,” *Plants*, vol. 14, no. 3, 2025, doi: 10.3390/plants14030488.
17. S. Peyghambarzadeh, T. Babaeinejad, J. Hadian, A. Fallah, and N. Ghanavati, “Growth and Phytochemical Properties of Horsetail Plant affected by Organic and Mineral Fertilization,” *Silicon*, vol. 15, no. 11, 2023, doi: 10.1007/s12633-023-02375-w.
18. F. Fauteux, F. Chain, F. Belzile, J. G. Menzies, and R. R. Bélanger, “The protective role of silicon in the Arabidopsis-powdery mildew pathosystem,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 103, no. 46, 2006, doi: 10.1073/pnas.0606330103.
19. D. Garcia, E. Garcia-Cela, A. J. Ramos, V. Sanchis, and S. Marín, “Mould growth and mycotoxin production as affected by Equisetum arvense and Stevia rebaudiana extracts,” *Food Control*, vol. 22, no. 8, 2011, doi: 10.1016/j.foodcont.2011.02.016.
20. A. Taylor *et al.*, “Equisetum arvense (horsetail) Extract: The First Approved Basic Substance Allowed for EU Crop Protection,” *International Journal of Bio-resource and Stress Management*, vol. 13, no. 6, 2022, doi: 10.23910/1.2022.2757.
21. R. S. Riseh, M. Ebrahimi-Zarandi, M. Gholizadeh Vazvani, and Y. A. Skorik, “Reducing drought stress in plants by encapsulating plant growth-promoting bacteria with polysaccharides,” 2021. doi: 10.3390/ijms222312979.
22. D. C. He, M. H. He, D. M. Amalin, W. Liu, D. G. Alvindia, and J. Zhan, “Biological control of plant diseases: An evolutionary and eco-economic consideration,” 2021. doi: 10.3390/pathogens10101311.
23. M. S. K. Anuar *et al.*, “Synergism: biocontrol agents and biostimulants in reducing abiotic and biotic stresses in crop,” 2023. doi: 10.1007/s11274-023-03579-3.
24. G. Franzoni, G. Cocetta, B. Prinsi, A. Ferrante, and L. Espen, “Biostimulants on Crops: Their Impact under Abiotic Stress Conditions,” 2022. doi: 10.3390/horticulturae8030189.
25. L. López-Serrano, L. Scalschi, R. Simeón, A. San Bautista, and A. I. González-Hernández, “Harnessing the Power of Biostimulants: A Comprehensive Review of Their Role in Enhancing Agricultural Productivity and Sustainability,” *Applied Sciences*, vol. 16, no. 4, p. 1924, Feb. 2026, doi: 10.3390/app16041924.
26. L. P. Canellas *et al.*, “Humic and fulvic acids as biostimulants in horticulture,” 2015. doi: 10.1016/j.scienta.2015.09.013.
27. E. Dutilloy, F. E. Oni, Q. Esmaeel, C. Clément, and E. A. Barka, “Plant Beneficial Bacteria as Bioprotectants against Wheat and Barley Diseases,” *Journal of Fungi*, vol. 8, no. 6, 2022, doi: 10.3390/jof8060632.

28. F. Yang, S. Jacobsen, H. J. L. Jørgensen, D. B. Collinge, B. Svensson, and C. Finnie, “Fusarium graminearum and its interactions with cereal heads: Studies in the proteomics era,” 2013. doi: 10.3389/fpls.2013.00037.
29. R. Mažylytė, J. Kaziūnienė, L. Orola, V. Valkovska, E. Lastauskienė, and A. Gegeckas, “Phosphate Solubilizing Microorganism Bacillus sp. MVY-004 and its Significance for Biomineral Fertilizers’ Development in Agrobiotechnology,” *Biology (Basel).*, vol. 11, no. 2, 2022, doi: 10.3390/biology11020254.
30. J. Kaziūnienė, R. Mažylytė, A. Krasauskas, M. Toleikienė, and A. Gegeckas, “Optimizing the Growth Conditions of the Selected Plant-Growth-Promoting Rhizobacteria Paenibacillus sp. MVY-024 for Industrial Scale Production,” *Biology (Basel).*, vol. 11, no. 5, 2022, doi: 10.3390/biology11050745.
31. G. Sezonov, D. Joseleau-Petit, and R. D’Ari, “Escherichia coli physiology in Luria-Bertani broth,” *J. Bacteriol.*, vol. 189, no. 23, 2007, doi: 10.1128/JB.01368-07.
32. A. K. Shukla, P. Vishwakarma, R. S. Singh, S. N. Upadhyay, and S. K. Dubey, “Bio-filtration of trichloroethylene using diazotrophic bacterial community,” *Bioresour. Technol.*, vol. 101, no. 7, 2010, doi: 10.1016/j.biortech.2009.10.094.
33. A. Tang, A. O. Haruna, N. M. A. Majid, and M. B. Jalloh, “Potential PGPR properties of cellulolytic, nitrogen-fixing, phosphate-solubilizing bacteria in rehabilitated tropical forest soil,” *Microorganisms*, vol. 8, no. 3, 2020, doi: 10.3390/microorganisms8030442.
34. S. Syafruddin *et al.*, “Rhizobacteria consortium as a biofertilizer to enhance yield, nutrient use, and fertilizer efficiency in maize,” *Bragantia*, vol. 84, 2025, doi: 10.1590/1678-4499.20240196.
35. B. N. Fitriatin, O. Mulyani, D. Herdiyantoro, T. A. Alahmadi, and M. Pellegrini, “Metabolic characterization of phosphate solubilizing microorganisms and their role in improving soil phosphate solubility, yield of upland rice (Oryza sativa L.), and phosphorus fertilizers efficiency,” *Front. Sustain. Food Syst.*, vol. 6, 2022, doi: 10.3389/fsufs.2022.1032708.
36. D. Paul and S. Narayan Sinha, “PHOSPHATE SOLUBILIZING ACTIVITY OF SOME BACTERIAL STRAINS ISOLATED FROM JUTE MILL EFFLUENT EXPOSED WATER OF RIVER GANGA,” *Indian Journal of Fundamental and Applied Life Sciences*, vol. 3, no. 3, 2013.
37. V. S. Meena *et al.*, “Potassium solubilizing rhizobacteria (KSR): Isolation, identification, and K-release dynamics from waste mica,” *Ecol. Eng.*, vol. 81, 2015, doi: 10.1016/j.ecoleng.2015.04.065.
38. D. Herdiyantoro *et al.*, “The ability of potassium solubilizing rhizo-bacteria isolated from maize rhizosphere for microbial fertilizer,” in *IOP Conference Series: Earth and Environmental Science*, 2018. doi: 10.1088/1755-1315/205/1/012011.
39. A. Duba, K. Goriewa-Duba, and U. Wachowska, “A review of the interactions between wheat and wheat pathogens: Zymoseptoria tritici, fusarium spp. and parastagonospora nodorum,” 2018. doi: 10.3390/ijms19041138.
40. S. Tewari and S. Sharma, “Rhizobial-metabolite based biocontrol of fusarium wilt in pigeon pea,” *Microb. Pathog.*, vol. 147, 2020, doi: 10.1016/j.micpath.2020.104278.
41. N. Xi *et al.*, “Identification of antagonistic activity against Fusarium, and liquid fermentation of biocontrol Bacillus isolated from wolfberry (Lycium barbarum) rhizosphere soil,” *Front. Microbiol.*, vol. 16, 2025, doi: 10.3389/fmicb.2025.1601945.
42. M. A. C. Latz, B. Jensen, D. B. Collinge, and H. J. Lyngs Jørgensen, “Identification of two endophytic fungi that control Septoria tritici blotch in the field, using a structured screening approach,” *Biological Control*, vol. 141, 2020, doi: 10.1016/j.biocontrol.2019.104128.
43. REBIB Hanene, “Biological control of Fusarium foot rot of wheat using fengycin-producing Bacillus subtilis isolated from salty soil,” *Afr. J. Biotechnol.*, vol. 11, no. 34, 2012, doi: 10.5897/ajb11.2887.
44. S. Jacksch *et al.*, “Influence of sampling site and other environmental factors on the bacterial community composition of domestic washing machines,” *Microorganisms*, vol. 8, no. 1, 2020, doi: 10.3390/microorganisms8010030.
45. A. Jaiswal, G. Kumari, V. K. Upadhyay, J. Pradhan, and H. S. K. Pramanik, “A methodology to develop liquid formulation of biofertilizer technology,” *The Pharma Innovation Journal* , vol. 12, no. 11, pp. 875–881, Sep. 2023.
46. S. S. Chompa *et al.*, “Survival of beneficial microbes in liquid bioformulation and optimization of different carrier materials using RSM technique,” *International Microbiology*, vol. 27, no. 3, 2024, doi: 10.1007/s10123-023-00423-4.

47. S. Hadian, D. L. Smith, and S. Supronienė, "Modulating the Plant Microbiome: Effects of Seed Inoculation with Endophytic Bacteria on Microbial Diversity and Growth Enhancement in Pea Plants," *Microorganisms*, vol. 13, no. 3, 2025, doi: 10.3390/microorganisms13030570.
48. J. Sinkevičienė, J. Pekarskas, and A. Krasauskas, "Biologinių produktų poveikis ekologinių žieminių kviečių sėklai," *Žemės ūkio mokslai*, vol. 22, no. 2, 2015, doi: 10.6001/zemesukiomokslai.v22i2.3112.
49. M. Liaqat, S. Riaz, I. Yahya, and M. Pervez, "Synthesis and application of biodegradable seed coatings to assess the infestation of chickpea (Cicer arietinum L.) seeds by pests," *Journal of the Saudi Society of Agricultural Sciences*, 2024, doi: 10.1016/j.jssas.2024.08.004.
50. "https://www.j3c-agri.com/produits-j3c."
51. "https://www.schacht.de/produkt/Bio-Pflanzenstaerkungsmittel-Brennnessel-_-Schafgarbe-1-Liter/ ."
52. P. Vyas, P. Rahi, and A. Gulati, "Stress tolerance and genetic variability of phosphate-solubilizing fluorescent Pseudomonas from the cold deserts of the trans-himalayas," *Microb. Ecol.*, vol. 58, no. 2, 2009, doi: 10.1007/s00248-009-9511-2.
53. H. Alattas, B. R. Glick, D. V. Murphy, and C. Scott, "Harnessing Pseudomonas spp. for sustainable plant crop protection," 2024. doi: 10.3389/fmicb.2024.1485197.
54. J. Sikorski, E. Stackebrandt, and W. Wackernagel, "Pseudomonas kilonensis sp. nov., a bacterium isolated from agricultural soil," 2001. doi: 10.1099/00207713-51-4-1549.
55. A. E. LaBauve and M. J. Wargo, "Growth and laboratory maintenance of Pseudomonas aeruginosa," *Curr. Protoc. Microbiol.*, no. SUPPL.25, 2012, doi: 10.1002/9780471729259.mc06e01s25.
56. Y. Yan *et al.*, "Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated Pseudomonas stutzeri A1501," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 105, no. 21, 2008, doi: 10.1073/pnas.0801093105.
57. E. Combes-Meynet, J. F. Pothier, Y. Moënne-Loccoz, and C. Prigent-Combaret, "The pseudomonas secondary metabolite 2,4-diacetylphloroglucinol is a signal inducing rhizoplane expression of azospirillum genes involved in plant-growth promotion," *Molecular Plant-Microbe Interactions*, vol. 24, no. 2, 2011, doi: 10.1094/MPMI-07-10-0148.
58. M. Duman *et al.*, "Pseudomonas piscium sp. Nov., pseudomonas pisciculturae sp. nov., pseudomonas mucoides sp. nov. and pseudomonas neuropathica sp. nov. isolated from rainbow trout," *Int. J. Syst. Evol. Microbiol.*, vol. 71, no. 3, 2021, doi: 10.1099/ijsem.0.004714.
59. M. C. Macián, T. Lucena, D. R. Arahal, M. A. Ruvira, R. Aznar, and M. J. Pujalte, "Pseudidiomarina piscicola sp. nov., isolated from cultured European seabass, Dicenthrarchus labrax," *Arch. Microbiol.*, vol. 203, no. 4, 2021, doi: 10.1007/s00203-020-02131-3.
60. S. Sanow *et al.*, "Molecular Mechanisms of Pseudomonas-Assisted Plant Nitrogen Uptake: Opportunities for Modern Agriculture," *Molecular Plant-Microbe Interactions*, vol. 36, no. 9, 2023, doi: 10.1094/MPMI-10-22-0223-CR.
61. M. Wang *et al.*, "Effect of the nitrogen-fixing bacterium Pseudomonas protegens CHA0-ΔretS-nif on garlic growth under different field conditions," *Ind. Crops Prod.*, vol. 145, 2020, doi: 10.1016/j.indcrop.2019.111982.
62. K. Mahmud, S. Makaju, R. Ibrahim, and A. Missaoui, "Current progress in nitrogen fixing plants and microbiome research," 2020. doi: 10.3390/plants9010097.
63. X. Chen *et al.*, "Genome-based identification of phosphate-solubilizing capacities of soil bacterial isolates," *AMB Express*, vol. 14, no. 1, 2024, doi: 10.1186/s13568-024-01745-w.
64. M. Safari, E. Motamedi, H. Kari Dolatabad, and S. A. M. Modarres Sanavy, "Nano-carriers effects on the viability and efficiency of Pseudomonas strains as phosphate solubilizing bacteria," *Heliyon*, vol. 6, no. 10, 2020, doi: 10.1016/j.heliyon.2020.e05076.
65. S. Ibietela, R. Renner, Y. Gote, and C. Author, "Isolation of bacteria from soil and water environments with phosphate solubilizing potential," *~ 145 ~ Journal of Advances in Microbiology Research*, vol. 5, no. 2, 2024.
66. P. P. N. Jabin and S. Ismail, "Solubilization of Insoluble Potassium by Different Microbial Isolates in vitro Condition," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 6, no. 10, 2017, doi: 10.20546/ijcmas.2017.610.424.
67. Y. Zhao *et al.*, "Isolation and Characterization of Potassium-Solubilizing Rhizobacteria (KSR) Promoting Cotton Growth in Saline–Sodic Regions," *Microorganisms*, vol. 12, no. 7, 2024, doi: 10.3390/microorganisms12071474.
68. Y. Liao, X. Xu, H. Peng, A. Chen, C. Hao, and C. Li, "Biocontrol Efficacy of Bacillus velezensis FXJ Against Fusarium graminearum-Induced Fusarium Head Blight in Wheat," *Journal of Fungi*, vol. 12, no. 1, 2026, doi: 10.3390/jof12010037.

69. N. Khaledi, P. Taheri, and M. Falahati-Rastegar, “Evaluation of resistance and the role of some defense responses in wheat cultivars to Fusarium head blight,” *J. Plant Prot. Res.*, vol. 57, no. 4, 2017, doi: 10.1515/jppr-2017-0054.
70. A. F. Mahmoud, “Genetic variation and biological control of Fusarium graminearum isolated from wheat in Assiut-Egypt,” *Plant Pathol. J. (Faisalabad).*, vol. 32, no. 2, 2016, doi: 10.5423/PPJ.OA.09.2015.0201.
71. N. Allioui, F. Driss, H. Dhouib, L. Jlail, S. Tounsi, and O. Frikha-Gargouri, “Two Novel Bacillus Strains (subtilis and simplex Species) with Promising Potential for the Biocontrol of Zymoseptoria tritici, the Causal Agent of Septoria Tritici Blotch of Wheat,” *Biomed Res. Int.*, vol. 2021, 2021, doi: 10.1155/2021/6611657.
72. R. Platel *et al.*, “Isolation and Identification of Lipopeptide-Producing Bacillus velezensis Strains from Wheat Phyllosphere with Anti-fungal Activity against the Wheat Pathogen Zymoseptoria tritici,” *Agronomy*, vol. 12, no. 1, 2022, doi: 10.3390/agronomy12010095.
73. R. L. Bargabus, N. K. Zidack, J. E. Sherwood, and B. J. Jacobsen, “Oxidative Burst Elicited by Bacillus mycoides Isolate Bac J, a Biological Control Agent, Occurs Independently of Hypersensitive Cell Death in Sugar Beet,” *Molecular Plant-Microbe Interactions*, vol. 16, no. 12, 2003, doi: 10.1094/MPMI.2003.16.12.1145.
74. H. Peng *et al.*, “Biocontrol characteristics and management potential of Paenibacillus polymyxa A818 against Fusarium head blight in wheat,” *BioControl*, Apr. 2026, doi: 10.1007/s10526-026-10401-0.
75. D. Torres-Püschel *et al.*, “Paenidepsins are a Family of Lipopeptides from *Paenibacillus*,” *J. Nat. Prod.*, vol. 89, no. 3, pp. 966–979, Mar. 2026, doi: 10.1021/acs.jnatprod.5c01579.
76. J. Dobrzyński and A. Naziębło, “Paenibacillus as a Biocontrol Agent for Fungal Phytopathogens: Is P. polymyxa the Only One Worth Attention?,” 2024. doi: 10.1007/s00248-024-02450-8.
77. N. A. Salih, “Antibacterial effect of nettle (Urtica dioica),” *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, vol. 13, no. 1, 2014, doi: 10.29079/vol13iss1art270.
78. Edris Mahdavi Fikjvar, Mehrnoush Ebadi, Rahim Ahmadi, and Nazanin Abroon, “Exploring the Antibacterial Effects of Urtica dioica Leaf Oil Extract against Escherichia coli and Staphylococcus aureus ,” *International Journal of BioLife Sciences* , vol. 4, no. 2, pp. 64–73, Apr. 2025.
79. P. Yuan, Z. Song, L. Zhang, Z. Pan, and X. Chen, “Exploring the Multifunctional Role of Paenibacillus Metabolites in Various Fields,” 2025. doi: 10.3390/ijms262412089.
80. G. Benjamin, G. Pandharikar, and P. Frendo, “Salicylic Acid in Plant Symbioses: Beyond Plant Pathogen Interactions,” 2022. doi: 10.3390/biology11060861.
81. Huda Jihad G. Al-shattraw, “Effect of Willow Bark Extract on The Activity of Gram-Negative Intestinal Bacteria: A Review,” *CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES*, vol. 6, no. 6, pp. 660–678, Mar. 2025.
82. M. Nagargade, V. Tyagi, and M. K. Singh, “Plant growth-promoting rhizobacteria: A biological approach toward the production of sustainable agriculture,” in *Role of Rhizospheric Microbes in Soil: Stress Management and Agricultural Sustainability*, vol. 1, 2018. doi: 10.1007/978-981-10-8402-7_8.
83. A. Pallag *et al.*, “Equisetum arvense L. extract induces antibacterial activity and modulates oxidative stress, inflammation, and apoptosis in endothelial vascular cells exposed to hyperosmotic stress,” *Oxid. Med. Cell. Longev.*, vol. 2018, 2018, doi: 10.1155/2018/3060525.
84. J. Dou *et al.*, “Willow Bark-Derived Material with Antibacterial and Antibiofilm Properties for Potential Wound Dressing Applications,” *J. Agric. Food Chem.*, vol. 71, no. 44, 2023, doi: 10.1021/acs.jafc.3c00849.
85. J. S. Patel, V. Selvaraj, P. More, R. Bahmani, T. Borza, and B. Prithiviraj, “A Plant Biostimulant from Ascophyllum nodosum Potentiates Plant Growth Promotion and Stress Protection Activity of Pseudomonas protegens CHA0,” *Plants*, vol. 12, no. 6, 2023, doi: 10.3390/plants12061208.
86. F. Ahmad, I. Ahmad, and M. S. Khan, “Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities,” *Microbiol. Res.*, vol. 163, no. 2, 2008, doi: 10.1016/j.micres.2006.04.001.
87. B. R. Glick, “Plant Growth-Promoting Bacteria: Mechanisms and Applications,” *Scientifica (Cairo).*, vol. 2012, 2012, doi: 10.6064/2012/963401.

88. H. P. Devkota *et al.*, "Stinging Nettle (Urtica dioica L.): Nutritional Composition, Bioactive Compounds, and Food Functional Properties," 2022. doi: 10.3390/molecules27165219.

89. A. Mahmood, O. C. Turgay, M. Farooq, and R. Hayat, "Seed biopriming with plant growth promoting rhizobacteria: a review," *FEMS Microbiol. Ecol.*, vol. 92, no. 8, p. fiw112, Aug. 2016, doi: 10.1093/femsec/fiw112.

90. A. M. Timofeeva, M. R. Galyamova, and S. E. Sedykh, "Plant Growth-Promoting Soil Bacteria: Nitrogen Fixation, Phosphate Solubilization, Siderophore Production, and Other Biological Activities," 2023. doi: 10.3390/plants12244074.

91. I. Julia, M. Oscar, L. Analía, J. Zocolo Guilherme, and L. Virginia, "Biofertilization with Macrocystis pyrifera algae extracts combined with PGPR-enhanced growth in Lactuca sativa seedlings," *J. Appl. Phycol.*, vol. 32, no. 6, 2020, doi: 10.1007/s10811-020-02202-4.

92. F. Xu *et al.*, "Auxin-Producing Pseudomonas Recruited by Root Flavonoids Increases Rice Rhizosheath Formation through the Bacterial Histidine Kinase Under Soil Drying," *Advanced Science*, vol. 12, no. 34, 2025, doi: 10.1002/advs.202500607.

93. K. Adesh *et al.*, "Evaluation of Pseudomonas isolates from wheat for some important plant growth promoting traits," *Afr. J. Microbiol. Res.*, vol. 8, no. 27, 2014, doi: 10.5897/ajmr2014.6711.

94. E. Ibrahim *et al.*, "Biocontrol Efficacy of Endophyte Pseudomonas poae to Alleviate Fusarium Seedling Blight by Refining the Morpho-Physiological Attributes of Wheat," *Plants*, vol. 12, no. 12, 2023, doi: 10.3390/plants12122277.

95. F. Pérez-Labrada *et al.*, "From Phytochemicals to Physiology: The Metabolic and Redox Effects of Botanical Extracts on Crops," *Plants*, vol. 15, no. 8, p. 1237, Apr. 2026, doi: 10.3390/plants15081237.

96. N. Ullah *et al.*, "Integrated Effect of Algal Biochar and Plant Growth Promoting Rhizobacteria on Physiology and Growth of Maize Under Deficit Irrigations," *J. Soil Sci. Plant Nutr.*, vol. 20, no. 2, 2020, doi: 10.1007/s42729-019-00112-0.