

Original Article

Development of a two-component biological system for soil bioremediation and fertility restoration and its pilot efficiency assessment

Desenvolvimento de um sistema biológico de dois componentes para biorremediação e restauração da fertilidade do solo e avaliação de sua eficiência em escala-piloto

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Abstract

This article discusses the development and experimental evaluation of a two-component biosystem for the bioremediation of contaminated soils and restoration of their fertility. The proposed system includes a combination of degradative microorganisms and an organomineral biocomponent that improve the physicochemical and biological properties of the soil. The study aims to reduce the level of toxic pollutants, increase soil biological activity, and restore agrochemical parameters necessary for sustainable land management. During pilot trials on prepared model soils before introducing the biosystem, control and experimental groups were established and *Phaseolus vulgaris* growth rates, changes in phytotoxicity levels, microbiological activity, and soil fertility indicators were assessed. The results demonstrate the high effectiveness of the developed two-component biosystem approach: a reduction in pollutant concentrations, i.e., an improvement in soil structure, and the most pronounced changes in pH were observed with AKKU-36 + *P. fluorescens* + *P. putida* +. It was found that pH values in the Epin-Extra variant changed within the range of 6.04–5.80, demonstrating a synergistic effect. The introduction of the two bacteria together with AKKU-36 and, if necessary, Epin-Extra demonstrated an increased ability to support maximum plant growth. The bacteria *Pseudomonas fluorescens* and *Pseudomonas putida* effectively degraded organic pollutants (DDT, Aktara); the seeds in the control variant did not germinate at all, while the other variants showed their bioindices at their level, which confirms their bioremediation activity. The proposed two-component biosystem can be considered a promising, environmentally friendly method for restoring disturbed lands, suitable for use in agriculture and for the environmental rehabilitation of areas affected by anthropogenic impacts.

Keywords: bioremediation, contaminated soil, reclamation, two-component biosystem, degradative microorganisms, soil microflora, phytotoxicity, erosion, land reclamation.

Resumo

Este artigo aborda o desenvolvimento e a avaliação experimental de um biosistema de dois componentes para a biorremediação de solos contaminados e a restauração de sua fertilidade. O sistema proposto inclui uma combinação de microrganismos degradadores e um biocomponente organomineral que melhora as propriedades físico-químicas e biológicas do solo. O estudo visa reduzir o nível de poluentes tóxicos, aumentar a atividade biológica do solo e restaurar parâmetros agroquímicos necessários ao manejo sustentável da terra. Durante testes-piloto realizados em solos-modelo preparados antes da introdução do biosistema, foram estabelecidos grupos de controle e experimentais, sendo avaliadas as taxas de crescimento de *Phaseolus vulgaris*, as alterações nos níveis de fitotoxicidade, a atividade microbiológica e os indicadores de fertilidade do solo. Os resultados demonstram a alta eficácia da abordagem do biosistema de dois componentes desenvolvido: observou-se redução nas concentrações de poluentes e melhoria na estrutura do solo, sendo as alterações mais acentuadas no pH verificadas com a combinação AKKU-36 + *P. fluorescens* + *P. putida*. Constatou-se que os valores de pH na variante com Epin-Extra variaram entre 6,04 e 5,80, demonstrando um efeito sinérgico. A introdução das duas bactérias em conjunto com o AKKU-36 e, quando necessário, com o Epin-Extra, demonstrou capacidade aumentada de promover o crescimento máximo das plantas. As bactérias *Pseudomonas fluorescens* e *Pseudomonas*

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putida degradaram eficazmente poluentes orgânicos (DDT, Aktara); as sementes na variante de controle não germinaram, enquanto as demais variantes apresentaram índices biológicos satisfatórios, confirmando sua atividade de biorremediação. O biosistema de dois componentes proposto pode ser considerado um método promissor e ecologicamente correto para a restauração de terras degradadas, adequado ao uso na agricultura e na reabilitação ambiental de áreas afetadas por impactos antropogênicos.

Palavras-chave: biorremediação, solo contaminado, recuperação, biosistema de dois componentes, microrganismos degradadores, microflora do solo, fitotoxicidade, erosão, recuperação de terras.

1. Introduction

Soil pollution with petroleum products, heavy metals, pesticides, and other toxicants is one of the most pressing environmental problems of our time. Intensive industrial activities, oil extraction and transportation, the use of agrochemicals, and the accumulation of industrial and household waste lead to soil degradation, decreased biological activity, and the deterioration of agroecological soil properties. As a result, nutrient circulation is disrupted, organic matter content decreases, and soil fertility declines, negatively impacting ecosystem stability and plant productivity (Mediastari and Jumintono, 2025; Wijerathne et al., 2026).

Traditional methods of cleaning contaminated soils, including mechanical cleaning, thermal treatment, and chemical detoxification, are often characterized by high costs, energy consumption, and the risk of secondary environmental contamination. Therefore, in recent years, special attention has been paid to the development of environmentally friendly and cost-effective bioremediation technologies based on the use of microorganisms, plants, and organic biopreparations. A promising area, being implemented under project OGRN BR24993105, is the development of two-component biosystems combining microbiological and organomineral components. These systems were proposed to experts for a utility model, with an assessment of their ability to not only accelerate the decomposition of pollutants but also restore the physicochemical and biological properties of the soil. Microorganisms ensure the biological decomposition of toxicants, reduce the toxicity of pollutants, and create favorable conditions for the development of soil microbiota, while the second component—an accelerator—complements the elements in the soil structure, improves conditions in the rhizosphere of the plant, and promotes growth. Despite significant research in the field of bioremediation, comprehensive restoration of soil fertility after remediation has not yet been studied. Evaluation of the synergistic effects of combined biosystems under actual pollution conditions and their impact on soil agrochemical parameters, enzymatic activity, and plant growth is relevant.

2. Literature Review

With regard to agriculture, microbiology, and biotechnology, as well as means and methods for stimulating plant growth, increasing their resistance to stress factors, and removing organic pollutants from soils using chemically active compounds and microorganisms,

research is currently needed to prevent global problems in accordance with the goals of SDG-2, SDG-12, SDG-13, and SDG-15, i.e., systematizing the conservation and use of ecosystems. In this regard, a two-component system has been proposed consisting of the chemical growth stimulant Akku-36 (dimethyl (1-hydroxy-1-phenylethyl) phosphonate) and the bacteria *Pseudomonas fluorescens* and *Pseudomonas putida*. The system increases germination, growth, and biomass of plants, and also breaks down soil insecticides (DDT, Aktara). The current state of the environment, including agricultural ecosystems, requires ongoing research and enhanced biotechnology. Technological solutions we used:

- Akku-36 and Epin-Extra—chemical growth stimulants;
- *P. fluorescens* and *P. putida*—bacteria with pathogenic and destructive activity against plants;
- Potential for bioremediation of soils containing organic pollutants (DDT, Aktara).

Such studies were motivated by the insufficient understanding of the synergistic interactions between the chemical stimulant and bacteria, low efficacy when used simultaneously, and incomplete studies of insecticide degradation rates.

The authors describe that the *Pseudomonas fluorescens* strain exhibits protective and stimulating activity for plants (Anohina et al., 2018; Egamberdiyev et al., 2025). A significant number of patent applications confirm the effectiveness of *Pseudomonas fluorescens* in agriculture. Russian Patent No. 2235771 describes a *Pseudomonas fluorescens* strain used to produce phytopathogenic antifungal and bacterial preparations (Tereshchenko, 2017). Furthermore, a number of patents describe microbial consortia, including *Pseudomonas fluorescens* and *Pseudomonas putida*, which are used for the biodegradation of organic pollutants and enhancing soil biological activity. These studies confirm the high potential of biodegrading microorganisms as components of bioremediation systems (Bovet et al., 2016; Đukić et al., 2007). Bacterial strains *Bacillus mojavensis* AOP29, *Pseudomonas azotoformans* KOP35, *Stenotrophomonas rhizophila* AOP9, and *Pseudomonas migulae* KOP88 were isolated from the phylloplane, rhizosphere, and soil under vegetative plants of winter soft wheat and demonstrated effectiveness (Afordoan'i, 2023; Srinivasa et al., 2015; Al Abdallah et al., 2026).

The plant growth promotion mechanisms and induction of systemic disease resistance by *P. fluorescens* have been considered as properties of Sarkar (Sarkar et al., 2022; Yeshimbetova et al., 2026). Rhizosphere bacteria such as *Pseudomonas fluorescens* possess recognized mechanisms

for phytohormone production and phytopathogen suppression, which promote plant growth and resistance under various growing conditions (Wang and Yang et al., 2024; Hanifah et al., 2023; Bani et al., 2025). Some *Pseudomonas putida* strains exhibit the ability to colonize the root system and synthesize phytohormones, making them promising bioproducts for sustainable agriculture (Costa-Gutierrez et al., 2022; Aboalroub, 2026). Furthermore, *P. putida* has significant potential for biodegrading complex organic compounds, making it useful for remediating contaminated soils (Angraeni, 2024; Abdel-Ghani et al., 2026). Bacterial degradation is considered a more efficient and environmentally friendly method for pesticide degradation compared to physical and chemical methods. This study demonstrated the isolation and detection of a bacterial strain capable of degrading this contaminant and tolerating high concentrations, the degradation mechanism of this strain, and the factors influencing the degradation process (Eltoukhy et al., 2020). Plant growth-promoting rhizobacteria (PGPR) offer sustainable ways to enhance crop tolerance to environmental stress, including heavy metal toxicity. Understanding their role in reducing such stressors is important for improving biotechnological strategies aimed at food security and sustainable agriculture. A Cd experiment on rice (*Oryza sativa* L.) was conducted to determine the effects of different levels of *Serratia marcescens* and *Pseudomonas fluorescens* [10 and 20 ppm], individually and/or together, on the morphophysiological and biochemical properties when rice was subjected to severe Cd stress [0 (no Cd stress) and 100 μ M]. The results of the study showed that high levels of Cd stress in soil significantly reduced plant growth and biomass, photosynthetic pigments, and gas exchange parameters ($p \leq 0.05$) (Alhaj Hamoud et al., 2025). Plant growth-promoting rhizobacteria (PGPRs) are of particular interest in agricultural biotechnology due to their stimulating and protective effects on plants. Among the various types of PGPRs, some strains of *Pseudomonas putida* combine remarkable properties, such as phytohormone synthesis, nutrient solubility, adaptation to various stress conditions, and the ability to perfectly protect roots (Costa-Gutierrez et al., 2022). The use of herbicides and insecticides tends to deteriorate soil health worldwide (Thompson and Pitt, 2003). In our studies, stress factors such as DDT and the use of Actara led to soil pollution. An aerobic Gram-negative bacterium, *Pseudomonas putida* is commonly found in soil and air. It is known for its unique metabolic ability to break down various complex organic materials, such as aquaculture pollutants. The role of *P. putida* in biocompatibility and the ability to degrade organic compounds, such as aromatic and aliphatic chemicals, are examined. The effectiveness was synthesized using a qualitative method describing the key genetic properties and metabolic pathways that enhance the efficiency of bacterial bioremediation. These results demonstrate that exploiting the diverse properties of bacteria is important for innovative bioprocesses that can produce sustainable products, which in turn contributes to biotechnology-based economic development and environmental sustainability (Nasirov et al., 2025; Abdalhamid et al., 2025). In the field of bioremediation, not only DDT but also the organic

pollutant Bisphenol A is an important organic chemical as an intermediate, final product, and inert component in the production of many important products, such as polycarbonate plastics, epoxy resins, fireproofing materials, food and beverage packaging material, and more. BPA is an endocrine disruptor that mimics estrogen function, causing damage to reproductive organs (Eltoukhy et al., 2020). Disadvantages of these methods include the complex, multi-step process, which makes raw material acquisition difficult. Furthermore, there is limited research on the synergy between bacteria and stimulants.

An analysis of global experience shows that the role of biological plant protection products is increasing in the context of environmental measures. Currently, SDG-12 products and the two-component system we propose are harmless to the environment and consumers, unlike chemical insecticides. The development and production of commercial agricultural biological products based on *Pseudomonas* are being effectively carried out in almost all developed countries (Lucy et al., 2004).

Siderophores produced by antibiotics and bacteria can play a significant role in the suppression of phytopathogens. Both mechanisms facilitate microbial antagonism and lead to induced resistance. These rhizobacteria are useful for the development of new inoculants. Analysis of the aforementioned patent sources suggests that the most promising direction is the development of complex formulations combining bacterial cultures and growth stimulants of the *Pseudomonas* genus. The results obtained in this study, AKKU-36 + *Pseudomonas fluorescens* + *P. putida*, demonstrate the high efficacy of the combination, consistent with current trends outlined in the patent literature.

It was found that the nature of the nitrogen atom substitution in phosphonopiperidols significantly influences the activity of phosphorus-containing compounds in the piperidine series. Unsubstituted phosphonopiperidols are less active than N-alkyl balalams.

The effect of growth accelerators (gibberellins, auxins) on the morphological parameters of peas was studied; it was found that their use increases plant height, leaf area, and yield by 20-30% (Vi and Liu, 2019). Bioavailability (amino acids, algae extracts) increases drought tolerance of beans, improves plant photosynthesis and water balance (Zhang et al., 2020). Humic substances improve the growth and nutrient absorption of *Phaseolus vulgaris*, promote better absorption of macro- and microelements, increase plant biomass, and improve the development of the root system (Yao et al., 2018). When growing using rhizobacteria, an improvement in soil structure was also observed, stimulating bean growth, increasing nitrogen fixation, and yield (Ibarra et al., 2020). The use of plant growth-promoting rhizobacteria (PGPR) is a promising approach to environmentally friendly enhancement of crop yields and their resistance to stress factors. Among the most studied PGPR representatives, particular attention has been paid to species of the genus *Pseudomonas*, including *Pseudomonas fluorescens* and *Pseudomonas putida*, which are capable of stimulating plant growth by synthesizing phytohormones, increasing nutrient availability, and inducing systemic resistance (Glick, 2012; Nague et al., 2026). The effectiveness of microbial inoculants can be enhanced when used

in combination with biologically active plant growth regulators. However, the interactions of such components can be complex and manifest as additive, synergistic, or antagonistic effects (Backer et al., 2018). Insecticides with their persistence and environmental behavior deserve special attention. Persistent organic pollutants, including DDT (dichlorodiphenyltrichloroethane), are characterized by high chemical stability, a long half-life, and the ability to bioaccumulate in soil and biological objects. In contrast, modern neonicotinoid insecticides, such as thiamethoxam (the active ingredient in Actara), have a systemic effect and degrade more quickly, but can affect plant physiology and soil microbiota (Amutenya et al., 2026).

Many patent studies have been conducted on wheat rice families, while very little research has been conducted on legume families. The purpose of this utility model is to expand the spectrum and range of effects of a two-component system for stimulating plant growth and soil bioremediation, as well as to help eliminate the aforementioned deficiencies.

Technical Result:

- Increased germination of pea plants (*Phaseolus vulgaris*), their subsequent growth and development;
- Two-component system (AKKU-36+);
- Synergistic effect on plant growth;
- Removal of organic pollutants and bioremediation;
- Increased plant resilience and promotion of soil restoration.

During the growing season, 24 seedlings were biometrically assessed. The study was conducted by the International Seed Testing Association (ISTA, 2023). The study followed the principles of seed physiology, technology, and quality control discussed in Copeland and McDonald (2001) textbook, "Principles of Seed Science and Technology". Seed germination in 250 ml jars was carried out in accordance with the described approaches. Plant growth, root and shoot length, and seedling biomass were assessed.

The effect of the Akku-36 stimulator on the morphological state of seedlings of different variants was analyzed (Baitasheva et al., 2024). Anatomical changes in some variants were analyzed (Mussina et al., 2018). The area of the *Phaseolus vulgaris* plot is 10 m². In the experiments, depending on the sowing time and the stages of seedling ontogenesis, phenological observations for each variant were carried out based on previous research by scientists (Baitasheva et al., 2024). The ecological state of the soil cover and meeting biotechnological requirements required guidance providing for a two-component system for stimulating plant growth and soil bioremediation using stimulants such as AKKU-36 and rhizosphere bacteria with destructive activity.

3. Materials and Methods

Phaseolus vulgaris L. was tested under controlled conditions using 250 ml disposable jars. Pre-contaminated soil samples were prepared in these jars. The experiment included 10 treatments: control, stress treatments (Aktara and dust), AKKU-36, bacterial inoculations (*Pseudomonas fluorescens* and *Pseudomonas putida*), their combinations, and combined treatment with Epin-Extra. Seeds were

treated with bacterial suspensions before sowing. Plants were grown under identical conditions in accordance with standard agronomic requirements (Gomez and Gomez, 1984). The following parameters were evaluated: germination (%), root length (cm), shoot length (cm), and fresh biomass (g). Statistical analysis of the data was performed using analysis of variance (ANOVA) within the general linear model (GLM). The model included the following factors: AKKU-36, bacterial treatment, stressful situations, and Epin-Extra. Particular attention was paid to the analysis of factor interactions to identify synergistic effects. A difference value of $P < 0.05$ was considered statistically significant (Montgomery, 2017).

4. Results Analysis

For the technical result, dimethyl (1-hydroxy-1-phenylethyl) phosphonate, coded AKKU-36, with the following formula in Figure 1, was proposed as a growth stimulant for *Phaseolus vulgaris*.

Component A: additional Epin-Extra at a low concentration (0.001%) of dimethyl (1-hydroxy-1-phenylethyl) phosphonate (hereinafter referred to as Accu-36).

Component B: viable bacterial cells:

- *Pseudomonas fluorescens*;
- Application of *Pseudomonas putida* at a concentration of 10 ko CFU/ml to *Phaseolus vulgaris* pre-sowing treatment increases germination.

The components of the two-component system are stored separately and mixed before use. The two-component system (Accu-36 + bacteria) significantly increases germination, root and shoot length, and plant biomass. The introduction of two bacteria together with Accu-36 and, if necessary, Epin-Extra provides the maximum stimulating effect. *Pseudomonas fluorescens* and *Pseudomonas putida* bacteria effectively degrade organic pollutants (DDT, Aktara), confirming their bioremediation activity. The system simultaneously addresses agronomic and environmental issues, improves plant resilience and productivity, and reduces soil toxicity.

The integrated system demonstrates synergy exceeding the expected combined effect of the components, which is the *Pseudomonas fluorescens*.

The developed biosystem demonstrated, a concentration of dimethyl (1-hydroxy-1-phenylethyl) phosphonate synthesized at the Interdisciplinary Center at the Kazakh National Women's Pedagogical University (Kazakhstan) was proposed as a growth accelerator (0.001%) under the

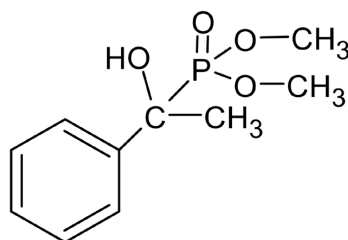


Figure 1. Dimethyl (1-hydroxy-1-phenylethyl) phosphonate.

code AKKU-36 (Dzhiembaev et al., 2023). I. According to the project of NAO “Kazakh National Research Technical University named after Satpayev” within the framework of the budget program 217 “development of science”, subprogram 101 “program-targeted financing of subjects of scientific and / or scientific and technical activity”: - 4 task of the project work “creation of a center for biotechnology R & D for the development and commercialization of agricultural products and technologies” OGRN BR24993105 within the framework of the task “development of effective technologies for restoring the fertility of disturbed soils by increasing the content of organic matter, ensuring the safety of cultivated products and preserving the fertile land fund of the Republic of Kazakhstan” is applied in the structure of the two-component system AKKU-36. Agriculture is the basis for the widespread use of soil resources and the application of biotechnology for its protection. Compared to the prototype, the proposed compound, which expands the spectrum and arsenal of effects of a two-component system on plant growth stimulation and soil bioremediation, is less toxic under experimental conditions, is virtually environmentally safe, and is a highly effective tool for increasing seedling yields and improving their quality. The Biological Research Laboratory at the Interdisciplinary Research Center of the Institute of Natural Science and the Department of Biology at the K. I. Satpayev Kazakh National Research Technical University conducted a study on the effects of *Phaseolus vulgaris* seeds on yield, growth, and development. The results of studies conducted using equipment acquired under Contract No. 990240005438/240554/00, based on the financial-economic justification, are presented.

4.1. Effect of a two-component (Accu-36 + bacteria) system on germination energy and viability of *Phaseolus vulgaris* seeds

The effects of various pea seed treatments on germination, root and shoot length, and plant biomass (Table 1) are shown at varying levels. Control plants without treatment showed minimal growth, indicating standard germination and development.

As a result of the study, it was found that treatment of *Phaseolus vulgaris* seeds with biological products significantly

affects their germination and morphophysiological indicators of seedlings. The results show a clear upward trend in all studied parameters compared to the control version (Figure 2).

The use of individual bacterial strains (*Pseudomonas fluorescens* and *Pseudomonas putida*) increased seed germination to 73-75%, exceeding the reference value (65%). This may be due to the ability of these microorganisms to synthesize phytohormones such as auxins, as well as improved nutrient availability in the rhizosphere.

A significant effect was observed with the combined treatment. Thus, the combination of AKKU-36 with the bacterial strains resulted in a 92% increase in germination, as well as a significant increase in root and shoot length. This indicates synergistic interactions that enhance the biological activity of the components.

The maximum values for all parameters were recorded with the combined treatment (AKKU-36 + *Pseudomonas fluorescens* + *Pseudomonas putida* + Epin-extra). Germination reached 95%, root length was 25.7 cm, shoot length was 20.5 cm, and net biomass was 7.70 g. This effect may be due to the combined action of the growth regulator, which activates physiological processes in microorganisms and the plant.

The results are consistent with literature data indicating that rhizobacteria of the genus *Pseudomonas* stimulate plant growth by producing biologically active substances, increasing stress resistance, and improving mineral nutrition.

Thus, the use of complex biopreparations is a promising approach to increasing crop yields.



Figure 2. The effect of treatment of *Phaseolus vulgaris* seeds with biological products on their germination and morphophysiological indicators of seedlings.

Table 1. Effect of two-component (Akku-36 + bacteria) system on germination energy and germination of *Phaseolus vulgaris* seeds.

No.	Processing options	Germination, %	Root Length, cm	Stem Length, cm	Pure biomass, G	Dry biomass, G
1	Control	65 ± 3	9.2 ± 0.3	10.1 ± 0.4	1.35 ± 0.05	0,350
2	AKKU-36	78 ± 2	11.6 ± 0.4	11.8 ± 0.5	2.10 ± 0.06	0,398
3	<i>Pseudomonas fluorescens</i>	75 ± 3	12.2 ± 0.3	11.4 ± 0.4	2.05 ± 0.05	0,402
4	<i>Pseudomonas putida</i>	73 ± 2	12.0 ± 0.3	11.2 ± 0.4	2.00 ± 0.04	0,414
5	AKKU-36 + <i>P. fluorescens</i>	88 ± 2	13.8 ± 0.4	13.1 ± 0.5	3.35 ± 0.07	0,635
6	AKKU-36 + <i>P. putida</i>	86 ± 2	12.6 ± 0.3	12.9 ± 0.5	3.30 ± 0.06	0,675
7	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i>	92 ± 2	24.5 ± 0.5	18.8 ± 0.6	7.50 ± 0.08	0.981
8	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i> + Epin-Extra	95 ± 1	25.7 ± 0.5	20.5 ± 0.6	7.70 ± 0.09	1,721

4.2. Experiment 1.1: controlling the effect of a two-component (Accu-36 + bacteria) system on *Phaseolus vulgaris* seed germination energy in a Petri dish in a laboratory

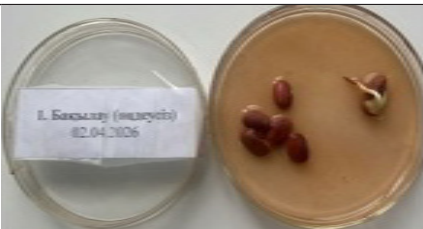
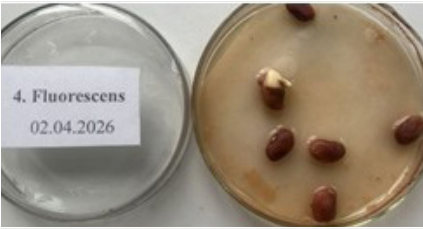
A laboratory Petri dish study was conducted in seven different variants, with six replicates over a 10-day period, using both the pure and two-component systems. Six seeds were placed in a dish, each with a germination rate of 12 percent (Table 2).

The overall germination rate of AKKU-36 + *P. fluorescens* + *P. putida* seeds was 98%, with a combined treatment of the two

bacteria. The AKKU-36 + *P. fluorescens* + *P. putida* + Epin-Extra treatment also achieved a 95% germination rate. Seeds treated with the AKKU-36 accelerator outperformed the control and *Pseudomonas fluorescens* variants by 76 ± 1 percent, while the Epin Extra variant outperformed them by 50 percent. Each seed was measured and divided into six average sizes.

The two-component system demonstrated a higher rooting rate than the other variants, 1.5 ± 0.5. In the experiment, the two-component system, i.e. with an accelerator, shows a synergistic effect between microorganisms, AKKU-36 +

Table 2. Effect of the two-component system on seed germination energy in a Petri dish.

No.	Processing options	Germination, %	Root length, cm, average	Appearance
1	Control	12 ± 3	0.5 ± 0.6	
2	AKKU-36	88 ± 2	0.9 ± 0.4	
3	<i>Pseudomonas fluorescens</i>	12 ± 3	0.33 ± 0.3	
4	<i>Pseudomonas putida</i>	0 ± 0	0 ± 0	
5	Epin-Extra	36 ± 2	0.7 ± 0.4	
6	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i>	98 ± 2	1.5 ± 0.5	
7	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i> + Epin-Extra	95 ± 1	1.0 ± 0.5	

P. fluorescens + *P. putida*, that is, it is observed in 98% with the combined treatment of two bacteria, and AKKU-36 + *P. fluorescens* + *P. putida* + Epin-Extra - germination reaches 95%, the root size is almost twice as large as that of the control, and increases root germination by 50%.

4.3. Study of bioremediation of contaminated soils

4.3.1. Study of bioremediation of contaminated soils using the *Phaseolus vulgaris* plant in a two-component (AKKU-36 + bacteria) system

The data presented in Table 3 also demonstrate the removal efficiency of DDT and Aktara insecticides from a soil sample using various treatment options, as well as the impact on plant growth.

The control soil exhibited virtually no insecticide loss—less than 10%—with low germination and biomass. Akku-36 itself had little effect on insecticide degradation. Bacterial treatment resulted in a significant reduction in DDT (up to 35%) and Aktara (up to 30%), confirming their destructive activity. The greatest effect was observed in the mixed Akku-36+ system, with both bacteria reducing DDT and Aktara levels in the soil by 60–70%, and significantly increasing plant germination and biomass.

The experiment was conducted on model soils contaminated with DDT and Aktara. The bacteria *Pseudomonas fluorescens* and *Pseudomonas putida* degrade insecticide residues (DDT, Aktara). They convert toxic compounds, organic soil pollutants, into less hazardous forms. The two-component system promotes the restoration of soil microbiological activity and improves its environmental safety. AKKU-36 + *P. fluorescens* + *P. putida* demonstrated a biomass of 1.40 ± 0.07 for *Phaseolus vulgaris* plants, while *P. fluorescens* + *P. putida* promotes the decomposition of organochlorine insecticides, including DDT, while the full-strength version demonstrates a biomass of 1.65 ± 0.08 , which enables the decomposition of neonicotinoid insecticides, including Aktara. The AKKU-36+ system, as shown in Table 2, demonstrated good results for both bacteria: a 70% reduction in DDT, a 60% reduction in Aktara, and a 40% increase in plant biomass. Finally, it was found that the claimed system provides biodegradation of persistent organic pollutants, including modern insecticides such as DDT and Aktara, which expands the scope of application of the invention to soil bioremediation tasks.

4.3.2. Growth analysis of *Phaseolus vulgaris* plants in a two-component (AKKU-36 + bacteria) system using stress factors

Stress factors, “Dust” (usually organochlorine insecticides (historically often DDT-like compounds), and Aktara were obtained for comparative analysis. Ten treatments, planted in quadruplicate, were seeded with 0.10 g of dry dust after 12 days. Aktara was dissolved in a five-liter volume and applied every seven days at 60 ml. The impact of steric factors was assessed based on the growth potential of *Phaseolus vulgaris* plants (Table 4).

Table 4 shows DDT → maximum phytotoxicity $25 \pm 2\%$ germination and 0.85 ± 0.05 biomass, and Aktara → average stress $55 \pm 3\%$ and 5.82 ± 0.03 g, bacteria + ACCU → overcoming toxicity 80 ± 2 , 81 ± 2 and 4.35 ± 0.07 , 4.30 ± 0.06 g, bacteria + ACCU Epin-extra → growth-promoting effect 91 ± 2 , 90 ± 1 and 5.50 ± 0.08 , 5.70 ± 0.09 g. This is a “stress-reaction-compensation system”, the observed differences in growth rates are associated not only with the presence of a stress factor, but also with its chemical nature and the rate of degradation of the soil environment. The most severe depression is associated with persistent organic pollutants, and modern insecticides cause reversible physiological changes that are partially compensated by biological agents.

4.3.3. Evaluation of the analysis of variance and interpretation of ANOVA stress factors for the growth of the *Phaseolus vulgaris* plant of a two-component (AKKU-36 + bacteria) system such as SPSS Output

In the experiment, the ANOVA factors were bacteria and DDT, and the interaction term was aktara. *Pseudomonas fluorescens* enhances mycorrhization and modulates the expression of antifungal defense response genes in the roots of aspen seedlings.

The ANOVA model we use is the General Linear Model (GLM) (Equation 1):

$$Y = AKKU + Bacteria + Stress + Epin Ekstra \times Bacteria \quad (1)$$

The main interaction here is (Equation 2):

$$AKKU \times Bacteria \rightarrow synergy \quad (2)$$

During the experiment, the treatments showed particularly significant differences in shoot growth of *Phaseolus vulgaris* plants (Table 5).

Table 3. Bioremediation of contaminated soils.

No	Processing options	DDT reduction, %	Aktara reduction, %	Germination, %	biomass reduction, g
1	Control	5 ± 2	8 ± 2	50 ± 3	0.70 ± 0.05
2	AKKU-36	10 ± 3	12 ± 3	60 ± 3	0.90 ± 0.05
3	Bacteria	35 ± 4	30 ± 4	70 ± 3	1.10 ± 0.06
4	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i>	60 ± 5	50 ± 5	82 ± 2	1.40 ± 0.07
5	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i> + Epin-Extra	70 ± 5	60 ± 5	90 ± 2	1.65 ± 0.08

Note: Data are presented as mean and standard error ($M \pm m$). Bacterial names are italicized according to International Biological Nomenclature (APA, 2020).

Table 4. The level of resistance to the effects of stress factors.

No	Processing options	Germination, %	Root length, cm,	length along the stems, cm	Biomass, g
1	Control	66 ± 3	7.2 ± 0.3	11.3 ± 0.4	7.48 ± 0.05
2	Control + Aktara	55 ± 3	5.2 ± 0.3	8.1 ± 0.3	5.82 ± 0.03
3	Control + DDT	25 ± 2	2.5 ± 0.2	5.1 ± 0.4	0.85 ± 0.05
4	AKKU-36	72 ± 2	11.6 ± 0.4	25.8 ± 0.5	4.10 ± 0.06
5	<i>Pseudomonas fluorescens</i>	70 ± 3	12.2 ± 0.3	18.8 ± 0.4	4.05 ± 0.05
6	<i>Pseudomonas putida</i>	68 ± 2	12.0 ± 0.3	20.2 ± 0.4	3.30 ± 0.04
7	AKKU-36 + <i>P. fluorescens</i>	80 ± 2	13.8 ± 0.4	23.4 ± 0.5	4.35 ± 0.07
8	AKKU-36 + <i>P. putida</i>	81 ± 2	12.6 ± 0.3	25.6 ± 0.5	4.30 ± 0.06
9	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i>	91 ± 2	14.5 ± 0.5	33.8 ± 0.6	5.50 ± 0.08
10	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i> + Epin-Extra	90 ± 1	15.7 ± 0.5	35.5 ± 0.6	5.70 ± 0.09

Note: Data are presented as mean and standard error (M ± m). Bacterial names are italicized according to International Biological Nomenclature (APA, 2020).

Table 5. Effect of stress treatment on shoot growth of *Phaseolus vulgaris* plants.

No	Processing options	length along the stem, cm
1	Control	11.3
2	Control + Aktara	8.1
3	Control + DDT	5.1
4	AKKU-36	25.8
5	<i>Pseudomonas fluorescens</i>	18.8
6	<i>Pseudomonas putida</i>	20.2
7	AKKU-36 + <i>P. fluorescens</i>	23.4
8	AKKU-36 + <i>P. putida</i>	25.6
9	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i>	33.8
10	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i> + Epin-Extra	35.5

Note: The Latin names of bacteria are italicized in accordance with the requirements of International Biological Nomenclature (APA, 2020).

In the DDT variant, as shown in the table, the plant shoot length was 5.1 cm. Compared to AKKU-36 + *P. fluorescens* + *P. putida* + Epin-Extra, i.e., in version 10, it increased by 30.4 cm, reaching 35.5 cm (Figure 3).

We analyzed the effect of AKKU-36 and bacteria interactions on shoot length in an interaction table (Table 6).

The information in this table demonstrates synergy (Anova interaction). Shoots in the data with AKKU-36 showed significantly higher rates than those without AKKU-36. AKKU-36 enhances the activity of the bacteria, indicating synergy (Figure 4).

An interaction analysis of the factors revealed that the response lines for the variants with and without Akku-36 were not parallel, indicating statistically significant interactions between the factors. The most pronounced effect was observed with the combined use of AKKU-36 and the bacterial inoculation, indicating a synergistic interaction. In this experiment, we observed

that stress factors inhibited growth and increased growth under the influence of AKKU-36 and bacteria, and also observed a positive effect of TP when using the two-component system with synergy (Table 7).

In an analysis of variance (ANOVA, SPSS logic), where df, F, and p are statistical measures of variation or deviation. In our experience, df are values that can vary within variants. The primary result of the F (F-statistic) test is the ratio of the variance between groups to the variance within groups, i.e., $F \rightarrow$ the value of significant differences between groups. P (p-value, significance level) is the probability of obtaining these (or higher) results if there are no differences:

* $p < 0.05 \rightarrow$ differences are generally considered statistically significant;

* $p \geq 0.05 \rightarrow$ no reason to believe the groups are different.

Thus, in our experiment, observing the df data structure, the strength of the F-differences, and the extent to which you can rely on P-differences from the table, the ANOVA analysis

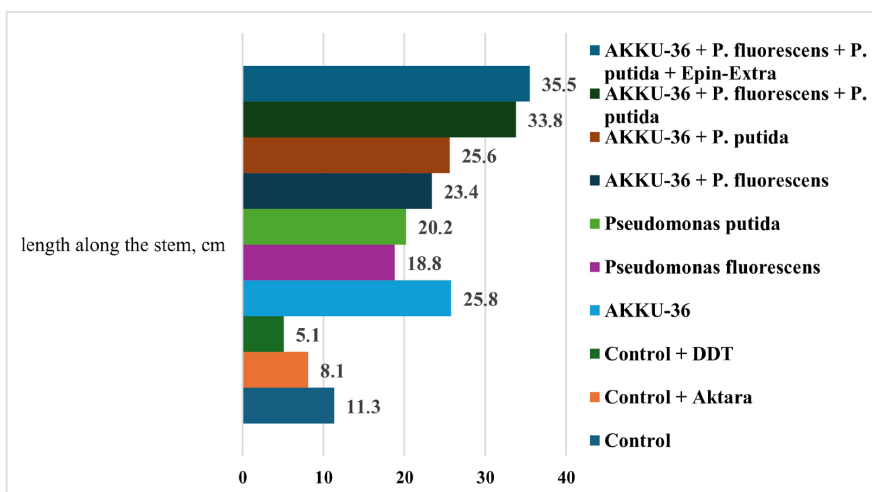


Figure 3. *Phaseolus vulgaris* L: effect on shoot growth.

Table 6. Interaction table.

No	Processing options	Without AKKU-36	With AKKU-36
1	Control	11.3	25.8
2	<i>P. fluorescens</i>	18.8	23.4
3	<i>P. putida</i>	20.2	25.6
4	Both	22.0 (average.)	33.8

Note: Bacterial abbreviations are italicized in accordance with International Biological Nomenclature requirements (APA, 2020).

showed that all main factors (Accu-36, bacterial inoculation, and stress conditions) had a statistically significant effect on bean growth rate ($p < 0.05$). The most pronounced effect was observed for the stress factor ($p < 0.001$), confirming the high sensitivity of *Phaseolus vulgaris* to chemical stress.

Analysis of variance (ANOVA) revealed statistically significant interactions between Accu-36 and bacterial transplant ($p = 0.001$) of all major factors are of particular importance, indicating the presence of a synergistic effect when used together.

4.4. The influence of a two-component (AKKU-36 + bacteria) system on the change in pH of model soils contaminated with pesticides

The experiment examined the effect of rhizosphere bacteria (*Pseudomonas fluorescens*, *Pseudomonas putida*) and growth accelerators (Accu-36, Epin-extra) on changes in the acidity of model soils contaminated with pesticides (DDT and Aktara) for growing *Phaseolus vulgaris*. In the model soil, 0.10 g of DDT (twice) was added to 250 ml of soil, dissolved in 5 liters of water at a concentration according to the Aktara instructions, and thoroughly mixed to ensure uniform distribution. Rhizobacteria treatment was carried out every 5 days with the preparation of a smoothie using 60 ml of distilled water. The accelerators were applied to *Phaseolus vulgaris* seeds in a container

the day before. The change in pH of the model soil on day 7 is shown in Table 8.

The experiment examined the effects of pesticide loading (DDT, Aktara), bacterial strains of the genus *Pseudomonas* (*Pseudomonas fluorescens*, *Pseudomonas putida*), and growth stimulants (AKKU-36, Epin-extra) on the acidity of the model soil during pea cultivation on the 7th day. The data presented in the table demonstrate a tendency toward gradual soil acidification due to rhizobacteria activity and the release of organic acids. Consequently, pH values on the 7th day were found to vary within the range of 6.04-6.33, corresponding to a slightly acidic pH. In the variants using bacterial preparations, a tendency toward a decrease in pH was noted due to the production of organic acids and the activation of microbiological processes in the rhizosphere. The use of growth stimulants enhanced this effect by increasing root exudates (Table 9).

In Table 9 data, there was a trend of 5.95 - 5.80 PH decrease on Day 21 compared to the control of a two-component system (6.36-6.39) in the variants of the introduction of bacteria (*Pseudomonas fluorescens*, *Pseudomonas putida*) (Figure 5).

This increased the production of organic acids (gluconic, acetic, etc.) by rhizobacteria and the mineralization of organic compounds, enhancing microbiological activity in the rhizosphere. In the scientists' studies, the ability of representatives of the genus *Pseudomonas* to actively alter and

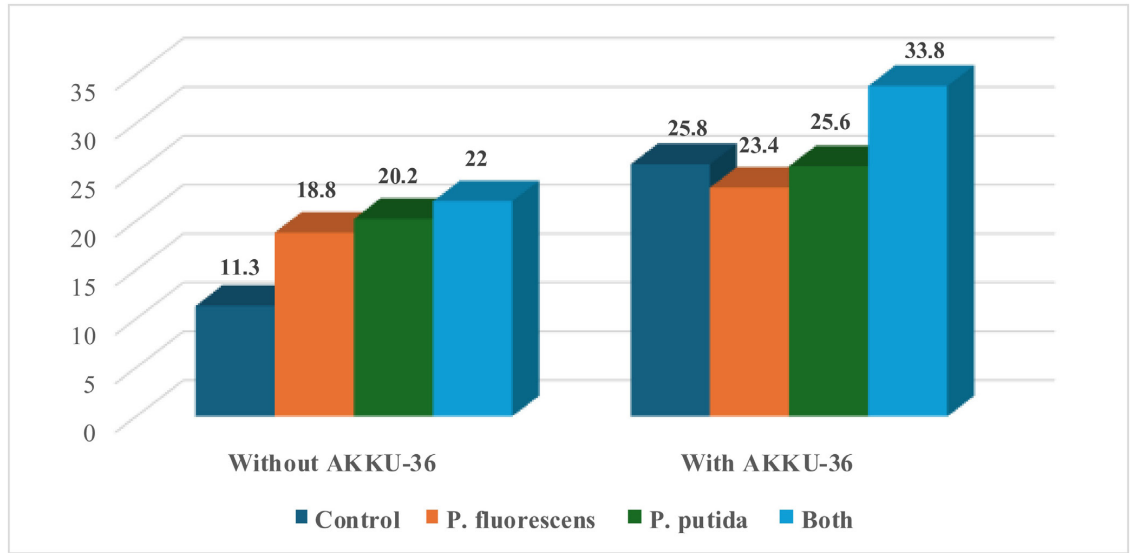


Figure 4. ANOVA interaction.

Table 7. Analysis of Variance (ANOVA, SPSS logic).

No	Processing options	df	F	p	Explanation
1	AKKU-36	1	85.4	<0.001	very important
2	Bacteria	3	41.2	<0.001	important
3	Stress (Aktara/Dust)	2	120.6	<0.001	very important
4	Epin-Extra	1	9.8	0.004	important
5	AKKU-36 × bacteria	3	18.7	0.001	SYNERGY
6	AKKU-36 × stress	2	22.3	<0.001	important
7	Bacteria × stress	6	7.5	0.01	moderately important
8	Error	-	-	-	-

Note: df is degrees of freedom; F is Fisher’s exact test; p is the level of statistical significance. Statistical parameters are italicized in accordance with APA style (APA, 2020).

Table 8. Change in pH of the model soil on the 7th day.

No	Processing options	7 күн
1	Control	6.21
2	Control + Aktara	6.29
3	Control + DDT	6.33
4	AKKU-36	6.04
5	Epin-Extra	6.09
6	<i>Pseudomonas fluorescens</i>	6.14
7	<i>Pseudomonas putida</i>	6.17
8	AKKU-36 + <i>P. fluorescens</i>	6.20
9	AKKU-36 + <i>P. putida</i>	6.21
10	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i>	6.13
11	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i> + Epin-Extra	6.04

Note: The names of microbiological objects (genera and species of *Pseudomonas*) in the experimental variants were written in italics in accordance with the International Biological Nomenclature (APA, 2020).

reduce the pH of the environment by synthesizing organic acids was used as the basis for increasing the availability of plant nutrients (Otieno et al., 2015). The use of stimulants (Accu-36, Epin-extra) weakly reduced the pH by increasing root system growth and the release of root exudates and activating the soil microbiota (up to 6.04-6.09). Egamberdieva et al., who found that phytohormones and growth stimulants increase microbial activity in the rhizosphere and lead to local acidification (Egamberdieva et al., 2016).

Results of AKKU-36 + *P. fluorescens* + *P. putida* + Epin-Extra showed that pH values varied in the range of 6.04-5.80, confirming the key role of rhizobacteria as a regulator of microbiological and biochemical processes in the soil and indicating the potential and synergy of using rhizobacteria for the bioremediation of contaminated soils. The DDT and Aktara control options reduced the deterioration of microbiota and the formation of organogenic acids. In the pesticide options (DDT and Aktara control), an increase in pH to 6.29-6.33 was observed. A number of studies have shown that contamination with organochlorine compounds (including DDT) reduces the biological activity of the soil, leading to the inhibition of microorganisms and changes in enzymatic activity, which leads to a change in pH (Cycoń et al., 2006).

On days 14 and 21, further changes in soil pH are expected depending on the activity of the rhizosphere microbiota and plant root secretions. It is assumed that treatments with *Pseudomonas* bacteria lower pH through the production of organic acids. Conversely, treatments with a pesticide load may have an elevated pH due to the inhibition of microbial activity. Maximum changes are expected with the combined use of bacteria and growth promoters. Vessey and Dawn, among other scientists, noted that the effect of PGPR depends on soil composition and buffering capacity (Vessey, 2003). In the two-component system treatments, lower pH values (6.04–5.80) compared to the control indicated the presence of soil buffering properties and a synergistic interaction. A Portable pH Meter PH-220 User Manual was used in the study.

The pesticide treatments have an elevated pH, which is associated with the inhibition of soil microbiota and reduced biological activity. Maximum changes in acidity are predicted with the combined use of bacteria and growth stimulants.

4.4.1. Two-component AKKU-36 + *P. fluorescens* + *P. putida* evaluation of synergy in the putida system

Table 10 data show the difference between the expected and actual combined treatment effects on the biomass of the *Phaseolus vulgaris* plant, based on the measurement units of the experiment to assess the effectiveness of insecticide disposal in Table 9.

AKKU-36 + *P. fluorescens* + *P. putida*, the expected effect was 1.20, real 1.40 → synergy + 0.20, while for the full system (Accu-36 + two bacteria + Epin-extra) the expected effect was 1.30, real 1.65 → synergy + 0.35. The use of an integrated

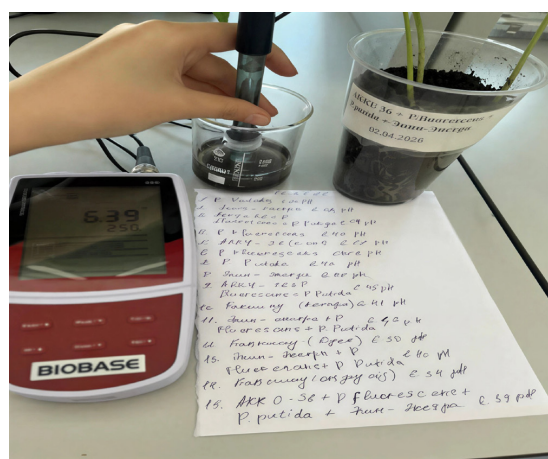


Figure 5. Measurement of the pH indicator of experimental versions with the Portable PH Meter PH-220 user Manual.

Table 9. Trend of pH change during triple inspection of model soil in the 7th day sequence.

No	Processing options	7 days	14 days	21 days	Trend
1	Control	6.21	6.18	6.15	weak ↓
2	Control + Aktara	6.29	6.32	6.36	↑
3	Control + DDT	6.33	6.35	6.45	↑
4	AKKU-36	6.04	5.98	5.92	↓
5	Epin-Extra	6.09	6.10	6.02	↓
6	<i>Pseudomonas fluorescens</i>	6.14	6.05	5.95	↓
7	<i>Pseudomonas putida</i>	6.17	6.08	5.98	↓
8	AKKU-36 + <i>P. fluorescens</i>	6.20	6.08	5.94	↓↓
9	AKKU-36 + <i>P. putida</i>	6.21	6.10	5.98	↓↓
10	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i>	6.13	6.03	5.92	↓↓↓
11	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i> + Epin-Extra	6.39	5.92	5.80	↓↓↓↓

Note: the scientific names of the bacteria were marked in italics in accordance with the APA standard (APA, 2020). There are no vertical lines in the table.

Table 10. Two-component AKKU - 36 + *P. fluorescens* + *P. synergy* in the putida system.

No	Processing options	Expected effect	real effect	synergism
1	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i>	1.20	1.40	+0.20
2	AKKU-36 + <i>P. fluorescens</i> + <i>P. putida</i> + Epin-Extra	1.30	1.65	+0.35

Note: abbreviated names of bacteria were italicized in accordance with the APA format (APA, 2020). Vertical border lines are not used in the table.

system provides a pronounced synergistic effect that exceeds the sum of the individual effects of each component, which confirms the effectiveness of the new invention.

5. Conclusion

The integrated system demonstrates a synergistic effect between the chemical stimulant and microorganisms. AKKU-36 + *P. fluorescens* + *P. putida* demonstrates a 92% synergistic effect with combined treatment of the two bacteria. AKKU-36 + *P. fluorescens* + *P. putida* + Epin-Extra results in a germination rate of 95% and biomass almost doubles compared to the control, significantly enhancing plant growth and development. In an experiment, a two-component system, i.e., one with an accelerator, demonstrated a synergistic effect between microorganisms. AKKU-36 + *P. fluorescens* + *P. putida* demonstrated a 98% synergistic effect with the combined treatment of the two bacteria. AKKU-36 + *P. fluorescens* + *P. putida* + Epin-Extra resulted in a 95% germination rate, almost twice the root size of the control, and increased root germination by 50%. The two-component system effectively performs a dual function, stimulating plant growth while simultaneously helping to remove toxic insecticides from the soil. The two-component system (Akku-36 + bacteria) significantly increases germination, root and shoot length, and plant biomass. The introduction of the two bacteria together with Akku-36 and, if necessary, Epin-Extra provides the maximum stimulating effect. The pH of the model soil was found to fluctuate between 6.04 and 6.39 on the 7th day, corresponding to a slightly acidic environment. *Pseudomonas* (*P. fluorescens*, *P. putida*) use leads to a decrease in soil pH, which is due to the production of organic acids and the activation of microbiological processes. The most obvious changes were pH-AKKU-36 + *P. fluorescens* + *P. putida* + Epin-Extra. It was found that in the pH-AKKU-36 variant, pH values varied in the range of 6.04-5.80, indicating a synergistic effect. *Pseudomonas fluorescens* and *Pseudomonas putida* bacteria effectively degrade organic pollutants (DDT, Aktara), confirming their bioremediation activity. The integrated system demonstrates synergy exceeding the expected combined effect of the components, which is the main evidence of the degree of novelty and ingenuity. The system simultaneously addresses agronomic and environmental issues, increases plant resilience and productivity, and reduces soil toxicity.

Data Availability Statement

Data will be available from the corresponding author upon reasonable request.

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