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# SEED-TREATED ENDOPHYTE BACTERIA-CHEMICAL CONSORTIUM POTENTIAL IN INHIBITING THE EFFECT OF *RHIZOCTONIA SOLANI* ON *PHASEOLUS VULGARIS*

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## SUMMARY

The concerned study aimed to evaluate the biological control of root rot disease of common beans (*Phaseolus vulgaris* L.), which causes considerable yield losses, using endophytic bacteria isolated from halo-xerophytic plants. The vegetative experiment revealed *P. vulgaris* plants have the potential to control virulence of the fungus *Rhizoctonia solani*, which causes root rot, based on consortia of endophytic bacteria and chemicals. The severity levels of plant disease, as determined, employed the damage scale method. Using the damaged background + conditional microbial preparation + Topaz (10 µg/ml), very light damage (1 ≤ 1; 10%) was evident in the common bean. By treating *P. vulgaris* with conditional microbial preparation, the yield gained a significant increase, and the phytopathogen damage level decreased. The study proved the potential of microbial preparations based on endophytic bacterial strains for inhibiting the effects of *R. solani* in common beans. These results can become initial data that could serve to expand the diversity of agents used against diseases in crop production.

**Keywords:** Bacteria, common bean (*P. vulgaris* L.), damage, endophyte, *Rhizoctonia solani*, seeds, severity levels, Topaz

**Key findings:** The biological control of *R. solani* in common beans (*P. vulgaris* L.) using bacterial strains isolated from halo-xerophytes has provided a unique solution. The results could help in the development of strategies for biological control and reduce the use of chemicals in crop production**.**

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## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a globally grown species of the genus *Phaseolus*, being widely adapted and grown in various environmental conditions. *P. vulgaris* has usually attracted greater attention due to its high nutritional and economic values, typically one of the most important food legumes worldwide (Sendi *et al*., 2019). *P. vulgaris* seeds contain many nutrients, such as vitamins, anthocyanins, flavonoids, carbohydrates, proteins, amino acids, polyphenols, and minerals (Akramov *et al*., 2025). The common bean appeared to be very sensitive to climate change, temperature fluctuations, water content, light intensity, biotic stress, fungal pathogens, as well as numerous insects and various nematodes, which can cause the highest yield losses (Ghoneem *et al*., 2023).

Plant pathogenic fungi, including *Rhizoctonia*, *Fusarium*, and *Alternaria*, cause various diseases (Axanbayev and Ismailov, 2022; Norboyev *et al*., 2024). Root rot and wilt diseases of *P. vulgaris*, caused by *R. solani*, have become the most devastating diseases (Akramov *et al*., 2022). The pathogen primarily attacks the underground parts of plants, such as seeds and roots, and later, aerial parts, including seedlings, stems, leaves, and fruits. Damping is the most common symptom of *Rhizoctonia* infection, characterized by the failure of heavily infected seeds to germinate and the occurrence of considerable yield losses (López-Olmos *et al*., 2005). In some countries, such as Iran, the root rot disease can cause yield losses of 3.8% to 76.0% in common beans (Derbalah *et al*., 2022).

These phytopathogenic fungi can comprise two division types based on transmission, with one transmitted through seeds and the other through roots, flowers, and fruits (Naseri, 2008). Biotic stresses that infect common beans through seed transmission entailed a 'seed-borne pathogens' description (Elwakil *et al*., 2009). Numerous phytopathogenic fungi originate from seeds, which can affect plant growth and development and cause considerable yield losses (Dell'Olmo *et al*., 2023). *R. solani* is the most important species in the genus *Rhizoctonia* and one of the soil-borne fungi with wide variations in cultural characteristics, properties, and virulence (Etebu and Nwauzoma, 2017). *R. solani* inflicts a wide range of plant diseases, including damping-off, brown rot, root rot, and stem rot, with varying degrees of virulence, depending on the plant species (Zrenner *et al*., 2020).

Rhizoctonia infection may increase in response to numerous environmental and management factors (Erlacher *et al*., 2014). The management factors that affect the Rhizoctonia inoculums in the soil consist of soil type and structure, crop rotation practices with Rhizoctonia hosts, tillage techniques, and soil microbial communities (Garrett *et al*., 2011). For controlling fungal diseases, the use of various methods applies (Anes *et al*., 2010). Accordingly, research on the potential use of plant secondary metabolites for disease control has been of greater importance (Elsharkawy and El-Sawy, 2015). Phytochemicals, such as terpenoids, phenols, alkaloids, and glucosides, have attained successful identification as a class of chemicals found in plants due to their long-standing use for pest control (Hamza *et al*., 2016; Rayimova *et al*., 2024).

Recently, plant extracts have attracted greater interest as alternatives to synthetic fungicides, with attempts being made to use these plant extracts in disease control strategies (Bazarov *et al*., 2024). In addition to improving the quality of the yield and supporting plant growth and development, the plant and seed treatments with biopreparations prevent further pollution of the ecosystem, which is highly valuable at present (Ruziev *et al*., 2024). Based on the above discussion, the concerned study aimed to determine the potential of a seed-treated 'endophyte bacteria-chemical' consortium in inhibiting the effect of *R. solani* on common beans (*P. vulgaris* L.).

**Table 1.** Characterization of endophytic bacteria collection strains.

| Strain name | Gene bank account number | Isolated plant | References |
|---|---|---|---|
| *Pseudomonas putida* KoPr129 | ON567222 | *Bassia prostrata* (L.) Beck | Axanbayev and Ismailov (2022) |
| *Priestia endophytica* KoPr131 | ON567223 | | |
| *Bacillus subtilis* CrEw1018 | ON567361 | *Kraschenninnikovia ceratoides* (L.) Gueldenst. | Akramov *et al*. (2022) |
| *Bacillus amyloliquefaciens* HAPH2 | MZ443975 | *Haloxylon ammodendron* (C.A.Mey.) Bunge ex Fenzl | Alikulov *et al*. (2023) |

**Figure 1.** *P. vulgaris* seedlings infected with *R. Solani.*

## MATERIALS AND METHODS

### Research object

For controlling root rot disease in the common bean (*P. vulgaris* L.), the endophytic bacterial strains isolated from some halo-xerophytic plants grown in arid regions of Uzbekistan were treatments used. The collection of endophytic bacterial strains remained in the Laboratory of Molecular Biotechnology, Samarkand State University, Samarkand, Uzbekistan (Figure 1, and Table 1).

### Isolation of *R. solani*

In determining the biotechnological effectiveness of the targeted bacterial biopreparation strains against *R. solani*, the study used the phytopathogenic *R. solani* for root rot disease in common beans (*P. vulgaris* L.). The strain analysis continued on its cultivation on the potato dextrose agar (PDA) medium (Alikulov *et al*., 2023). For this, placing a 7-mm fungal disk in the center of the PDA medium had an inverted position. Incubation took place at 28 °C for 7–10 days.

### Determination of CFU (colony-forming unit)

After the incubation period, an inoculum preparation continued from the *R. solani*. The fungal mycelia grown in a Petri dish underwent separation from the substrate with a sterile spatula, with a suspension prepared by placing them in a test tube with sterile water. Taking the prepared suspension at 10 µl reached placement into a Goryaev chamber before counting (Sharma *et al*., 2021).

### Soil treatment

The soil obtained for the experiment sustained rigorous sterilization to create a controlled environment and eliminate indigenous microflora. The treatment proceeded to prevent any interference from existing soil pathogens or competing microorganisms during the experimental period. The 10–15 g

per kilogram of soil was successful in its addition to the prepared inoculum. Then, incubating the mixed soil after being placed in a plastic container took 2–3 days (the fungus adapts), requiring the temperature at 24 °C–28 °C and humidity at 70%–90%. Afterward, sowing the plant seeds ensued (Sharma *et al*., 2021).

**Seed treatment**

Common bean seed samples received surface sterilization in 1% NaOCl solution (for 4 minutes) before washing twice with sterilized distilled water and drying on sterilized filter papers at room temperature (18 °C–20 °C). The strains in the bacterial biopreparation were in a ratio of 1:1:1:1, with a 20% solution prepared by diluting the bacterial suspension to $10^8$ on *P. vulgaris* seeds and treating them for two hours. Topaz application, a drug rich in antifungal chemical compounds, served as a control. Topaz chemical drug succeeded in mixing at 0.25 ml per 100 ml of water before its treatment of seeds.

**Chemical drug test**

The chemical Topaz drug reached evaluation for the synergistic effect on the bacterial strains (Syngenta Crop Protection AG, Switzerland). In this case, the use of the LB solid nutrient medium served as a test. The said method's testing employed the Cross-streak method (Velho-Pereira and Kamat, 2011). In this case, the working solution prepared continued by mixing an average of 5 ml of the drug with 10 l of water.

**Assessment of disease severity**

During the 35-40-day growing season, the development of the plant and the stages of disease encountered assessment on a 0–5 scale during the infection period (Yang *et al*., 2014).
Disease severity scale:
5 ≥ 81 (100%) Very severe damage, with the root completely rotted, and the plant dries out;
4 ≥ 51 (80%) Severe damage, with the root surface rotted, the stem growth slowed down, and the leaves turned yellow;
3 ≥ 31 (50%) Moderate, more than mild damage;
2 ≥ 11 (30%) Mild damage;1 ≤ 1 (10%) Very mild damage and almost no signs of the disease; and
0 – No signs of damage.

**Antimicrobial preparation testing under field conditions**

For field tests, common bean (*P. vulgaris* L.) seeds received treatment with a bacterial biopreparation for two hours for the experimental variant and with water for the first control variant before planting. As a second control variant, common bean seeds without treatment used water instead. The strains used in the bacterial biopreparation were in the ratio of 1:1:1:1, diluting a 20% solution of the bacterial suspension to $10^8$ for *P. vulgaris* seeds. After treatment, the common bean seeds remained in the dark for 24 h to ensure sufficient inoculation. The used rows totaled 75 in agrotechnical work, with a row length of 200 m, a width of 0.6 m, and the average seed density was 8–10 seeds per 15–20 cm interval. The agrotechnical work proceeded on an area of 9000 $m^2$ (39°32'38.8"N 67°15'06.6"E). Productivity analysis included the total number of stems per plant, the number of productive stems, stem length, root mass at 30 cm of soil, 500 grain weight, and fungal infection rate (Karima and Samah, 2024).

**Data analysis**

The recorded data based on various parameters entailed analysis following the methodology of Lakin (1990). The data processed values received graphical representation using the Origin Pro (2024) program. The arithmetic means of the data obtained in 3–5 repeated experiments sustained further evaluation using a statistical significance level of $p \leq 0.05$.

## RESULTS

### *R. solani* virulence

Common bean (*P. vulgaris* L.) seeds, treated with a 20% solution of the bacterial suspension diluted to $10^{-8}$, lasted for two hours with the target bacterial biopreparation strains. In the infected soil, the average colony-forming unit (CFU) of the *R. solani* appeared to be 1.4 × $10^8$ mycelium/ml in 450–500 g of soil.

Topaz has the main purpose of combating plant pathogenic fungi. Based on the test results, the chemical preparation obtained no evaluation as a limiting factor in the growth and development of bacteria. Based on the results, it was possible to use the chemical preparation together with bacterial strains by integrating them.

The presented study resulted in selecting four variants, with two variants selected for the control, using 30 seeds for each variant. Water and the chemical preparation Topaz served for the control. The seeds sown in infected soil treated with *R. solani* underwent observations carried out for 35-40 days.

As a result of the first 10 days of observations, 28–29 seeds germinated in the Control + $H_2O$ and Control + Topaz variants, while 1–2 seeds died. In the experimental variants, the highest seed germination resulted in the variant with the harmful background + bacterial preparation + Topaz 10 µg/ml. In total, 27 seeds germinated, while three seeds died.

As a result of 25–30 days of observation, the number of surviving seedlings in the Control + $H_2O$ and Control + Topaz variants was 29–28. The number of seedlings that became sick and died was 1–2. In the variants infected with *R. solani*, the highest survival rate surfaced in the variant of damaged background + bacterial preparation + Topaz 10 µg/ml, and the number of surviving seedlings was 25. The number of dead seedlings was three, and the number of infected seedlings was two.

The formation of yellow spots on the leaves of infected seedlings of common bean and yellow spots on the stems were signs of root rot. The number of germinated, dead, and infected seeds in the remaining variants appears in Table 2. The lower values of the variants, viz., damaged background + $H_2O$, damaged background + bacterial preparation, and damaged background + Topaz 50 µg/ml, compared with the control variants, can be because of the highest content of *R. solani* CFU in the soil. This leads to reduced germination of seeds and the death and survival rate of the seedlings.

**Table 2**. Virulence of *R. solani* on *P. vulgaris.*

| Experience options | Number of germinated seeds | Number of seeds that died | Number of surviving seedlings | Number of diseased seedlings | Disease severity (score 1–5) |
|---|---|---|---|---|---|
| Control + $H_2O$ | 29 | 1 | 29 | 0 | 0 |
| Control + Topaz | 28 | 2 | 28 | 0 | 0 |
| Infected background + $H_2O$ | 4 | 26 | 0 | 4 | 5 ≥ 81% |
| Infected background + Conditional microbial preparation | 18 | 12 | 11 | 7 | 4 ≥ 51% |
| Infected background + Topaz 50 µg/ml | 23 | 7 | 19 | 4 | 3 ≥ 31% |
| Infected background + Conditional microbial preparation + Topaz 10 µg/ml | 27 | 3 | 25 | 2 | 1 ≤ 10% |

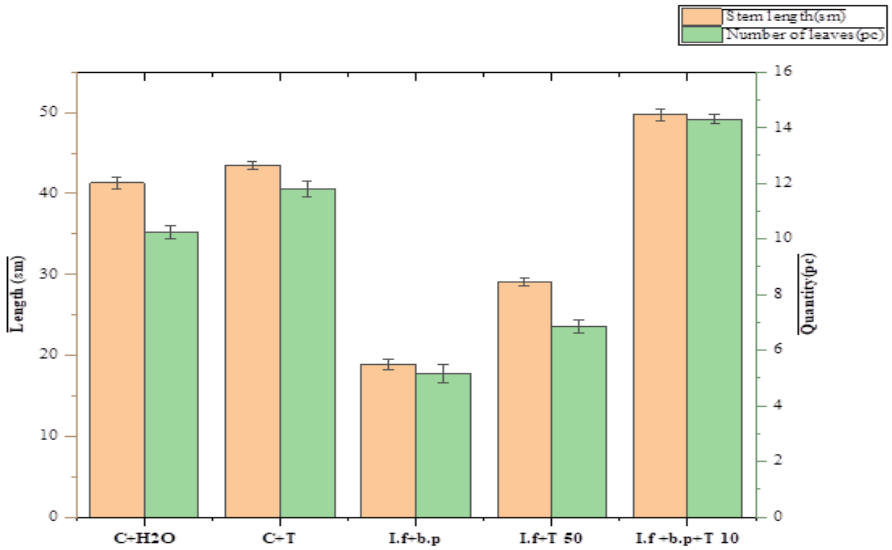


**Figure 2**. Effect of treatment with the 'endophyte bacteria-chemical substance' consortium on some biometric parameters of *P. vulgaris*: C+$H_2O$ (Control + $H_2O$); C+T (Control + Topaz); I.f+b.p (Infected fon + conditional microbial preparation); I.f+T50 (Infected fon + Topaz 50 mkg/ml); I.f+b.p+T10 (Infected fon + conditional microbial preparation + Topaz 10 mkg/ml), n=3, $p \leq 0.05$.

**Productivity traits of *P. vulgaris***

As a result of the virulence of *R. solani*, the results revealed the biometric traits of the common bean plant, as the *P. vulgaris* develops, were different in the experimental and control variants. The results enunciated that the stem length in the variants of Control + $H_2O$ and Control + Topaz occurred to be 48.7 ± 0.72 and 43.5 ± 0.46 cm, respectively. In the variants, viz., damaged background + bacterial preparation, damaged background + Topaz 50 µg/ml, and damaged background + bacterial preparation + Topaz 10 µg/ml, the stem length appeared to be 18.9 ± 0.70, 29.1 ± 0.51, and 49.7 ± 0.72 cm, respectively. The stem length in the variant damaged background + bacterial preparation + Topaz 10 µg/ml emerged to be 1.2–1.7 times longer than the control and other variants.

The number of leaves in the variants of Control + $H_2O$ and Control + Topaz was notable to be 10.24 ± 0.23 and 11.8 ± 0.28, respectively. The number of leaves in the variants damaged background + bacterial preparation, damaged background + Topaz 50 µg/ml, and damaged background + bacterial preparation + Topaz 10 µg/ml amounted to 5.16 ± 0.31, 6.84 ± 0.23, and 14.3 ± 0.17, respectively. The number of leaves in the variant damaged background + bacterial preparation + Topaz 10 µg/ml emerged to be 1.25–1.28 times higher than the control and other variants (Figure 2).

The wet weight of the common bean plants in the Control + $H_2O$ and Control + Topaz variants was 26.01 ± 0.41 and 28.17 ± 0.58 g, respectively, while the highest values for the said trait resulted in the variant damaged background + bacterial preparation + Topaz 10 µg/ml (29.34 ± 0.62 g). In the experimental variant, the wet plant weight was 1.1 times higher than the control variants. The dry weight of the plants in the Control + $H_2O$ and Control + Topaz variants were 8.52 ± 0.16 and 9.32 ± 0.24 g, respectively. The recorded maximum dry plant weight was in the variant damaged background + bacterial preparation + Topaz 10 µg/ml (10.43 ± 0.38 g), found to be higher than the control variants. In the variant of the damaged background + bacterial preparation + Topaz 10 µg/ml, it was evident that the stem length, the number of leaves, and wet and dry weights of plants were higher

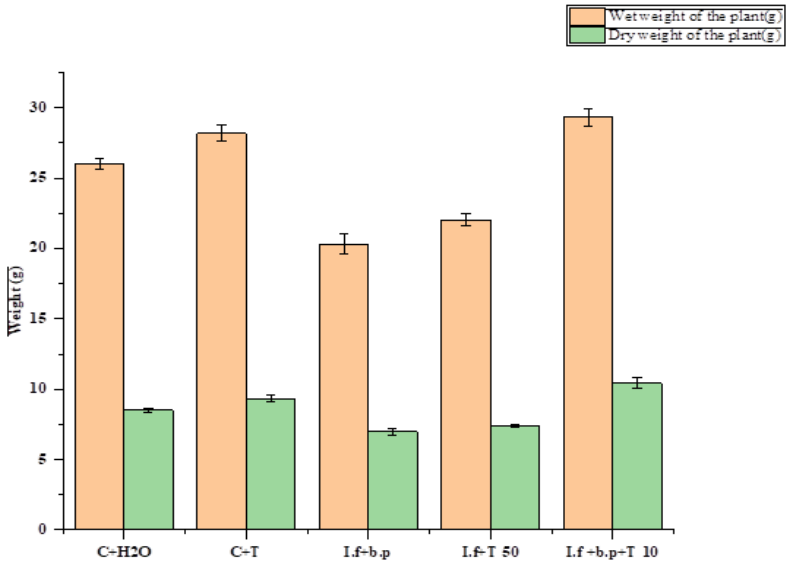


**Figure 3**. Effect of treatment with 'endophyte bacteria-chemical consortium' on *P. vulgaris* biomass productivity: C+$H_2O$ (Control + $H_2O$); C+T (Control + Topaz); I.f+b.p (Infected fon + conditional microbial preparation); I.f+T50 (Infected fon + Topaz 50 mkg/ml); I.f+b.p+T10 (Infected fon + conditional microbial preparation + Topaz 10 mkg/ml), n=3, $p \leq 0.05$.

than in the other variants (Figure 3). The higher values of plant size at different levels in the experimental variant versus control variants can be due to the positive effect of the combined bacterial preparation and Topaz chemical substance applied to the damaged soil.

According to the disease scale during the vegetation period of the common bean, as a result of the virulence of the *R. solani*, the highest disease rate resulted in the variant damaged background + $H_2O$ (5). It showed 81% of the plants as infected and dead in this variant. In the variant of damaged background + bacterial preparation, the disease scale reached an estimate of 4, with 51% of the plants infected and dead. In the variant damaged background + Topaz 50 µg/ml, the disease scale determined was 3, and 31% of the plants displayed infections. For the variant damaged background + bacterial preparation + Topaz 10 µg/ml, the estimated disease scale was 1, and only 10% of the common bean plants became infected and died. Meanwhile, in the control variants, soil used had no infection with *R. solani*. In the experimental variants, cases of disease and death were evident due to the virulence of *R. solani*, resulting in the rotting of plant roots and the formation of white-yellow symptoms on leaves of the common bean.

The field experiment took place in 2025 at the 'Islom Orzu' farm in the Taylak District of the Samarkand Region, Uzbekistan (39°32'38.8"N 67°15'06.6"E). The common bean (*P. vulgaris* L.) had farmers widely planting it as a second crop in this area, and to obtain higher yields, the use of chemical pesticides mainly rose. However, in the presented experiment, the use of chemical pesticides exhibited a significant decrease after the treatment with a bacterial preparation.

According to the results of the field experiment, the total number of stems per 1 $m^2$ of the common bean (*P. vulgaris*) was 288.2 ± 16.1 in the untreated (Control 1) and water treatment (Control 2) area, 296.2 ± 17.2 in the treated (C2) water treatment, and 327.4 ± 21.7 in the biopreparation (20%, 2 h). In this case, the number of stems was 1.10–1.12 times higher by treating with a biopreparation. The number of productive stems in the variant with a biopreparation was 1.17 times higher than C1 and C2, the stem

**Table 3**. Biometric parameters of *P. vulgaris* treated with a conditional microbial preparation (n = 5).

| Indicators | Control | | Experience | |
|---|---|---|---|---|
| | Dry (C1) | Water-treated (C2) | Conditional microbial preparation (20%, 2 hours) | Efficiency |
| Total number of stems ($m^2$) | 288.2±16.1 | 296.2±17.2 | 327.4±21.7* | 1.12 |
| Number of productive stems ($m^2$) | 176.5±9.2 | 188.6±12.3 | 214.4±14.2* | 1.17 |
| Stem length (cm) | 58.4±3.7 | 61.2±3.8 | 68.3±5.1* | 1.14 |
| Root mass in 30 cm soil layer (g) | 215.5±7.8 | 237.0±12.6 | 317.2±13.2* | 1.40 |
| 50 grain mass (g) | 148.2±8.4 | 157.8±9.6 | 198.2±12.3* | 1.30 |
| Yield ($g/m^2$) | 214.6±11.2 | 227.4±12.4 | 274.2±21.3* | 1.24 |
| Additional yield (relative to control) ($g/m^2$) | - | $C_1$+12.8 | $C_1$+59.6<br>$C_2$+46.8 | |
| Fungal infection rate (%) | 34 | 32 | 23 | -9 |

Note: *$P \leq 0.05$.

length was 1.14 times higher than C1 and C2, and the 500-grain weight was 1.3 times higher than C1 and C2. Moreover, the yield was 1.24 times higher than C1 and C2, and the additional yield was 59.0 and 46.8 times higher in the experimental variants than in C1 and C2. The level of fungal infection, as determined, counted the number of scab symptoms in 100 roots taken per 1 $m^2$ of area (Table 3). The higher yield as a result of treatment with a biopreparation compared with the control variants led to the decreased dependency on chemical preparations.

## DISCUSSION

In this study, the soil sustained treatment with the phytopathogenic *R. solani*. In preventing root rot disease in common beans (*P. vulgaris* L.) due to the virulence of *R. solani*, the use of a bacterial biopreparation and Topaz chemical preparation in the same environment and field experiments was successful. According to the results, the incidence rate in the experimental variant can reach 23%. It was evident that in the bacterial biopreparation, the number of germinated seeds, stem length, the number of leaves, and wet and dry mass of plants were higher than the control and other variants. Sangiogo *et al*. (2018) also reported that the treatment of common bean plants with a *B. cereus* strain in soil infected with *R. solani* provided a 37% reduction in root rot disease. Giorgio *et al*. (2016) found that *Pseudomonas brassicacearum*, *Bacillus megaterium,* and *Pseudomonas putida* strains proved more effective in preventing the disease.

In this study, the use of four bacterial strains resulted in a 70%–80% success in preventing the disease. Past findings reported the use of three different bacterial strains in equal amounts provided a higher disease prevention rate and increased yield to some extent in field experiments (Giorgio *et al*., 2016). Hagerty *et al*.'s (2015) findings showed seed germination was 13.33%, and seedling emergence during the growing season was 48.89%. In this case, the disease incidence was higher from the seedling stage to the flowering stage, given that the plants showed more susceptibility at this stage (Hagerty *et al*., 2015).

The results showed the pathogen infects at the seedling stage and persists throughout the vegetative and reproductive growth stages. The findings further revealed the root rot disease varies depending on the planting order, plant density, soil moisture, planting date and method, and crop variety. These considerable differences in the spread of the disease refer to the fact that these variations exhibited an association with changes in the crop growth stages (Tashpulatov *et al*., 2024).

In the common bean field experiments, a discovery indicated the bean yield obtained with a bacterial biopreparation was considerably higher than the control variants. In the bacterial biopreparation variant, the

disease rate also expressed a reduction of 10 times compared with the control variants, with the disease incidence also significantly reduced. Based on such results, one can conclude that the increase in productivity was due to a certain replacement of chemical preparations with biological preparations.

## CONCLUSIONS

In this study, on common beans (*P. vulgaris* L.), we noted the use of a 1:1:1:1 mixture of culture fluids of bacterial strains *P. putida* KoPr129, *P. endophytica* KoPr131, *B. subtilis* CrEw1018, and *B. amyloliquefaciens* HAPH2 isolated from halo-xerophyte plants and used as a microbial preparation increased the resistance properties to *R. solani.* Compared with control variants, separately and in combination with chemical agents, the mixture was favorable. In the variant of infected background + microbial preparation + Topaz 10 µg/ml, the percentage of infected crops decreased by 50% compared with the infected background + $H_2O$. For the variant of infected background + microbial preparation + Topaz 10 µg/ml, the common bean plants emerged with the best root length, leaf number, and biomass weight. In field experiments, outcomes proved that treating crops with a conditional microbial preparation improved the biometric indicators that determine crop yield. The results showed the importance of preventing various diseases by replacing chemical preparations with particular biological preparations in crop production.

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