










Effects of chemical and biological phosphorus fertilizers on the activity of antioxidant enzymes and some biochemical traits of cumin

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ABSTRACT

In this research on the effect of chemical and biological phosphorus fertilizers on antioxidant enzyme activity and biochemical characteristics of cumin, a factorial experiment was conducted in a complete randomized design over three replications in pot experiments. Treatment consisted of three levels of triple superphosphate chemical fertilizer (0, 50, and 100 kg ha⁻¹) and two levels of biofertilizer (no inoculation and inoculation with *Pseudomonas fluorescens* and *Bacillus subtilis* bacteria). The activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) and biochemical parameters like total chlorophyll, proline, and malondialdehyde (MDA) were quantified at the flowering stage. The results indicated that the combined treatment of 50% chemical fertilizer + biofertilizer possessed the maximum antioxidant enzyme activity; therefore, the CAT, POD, and SOD activities were increased by 42%, 35%, and 28% respectively compared to the control ($p < 0.05$). Moreover, the total chlorophyll content in the treatment was 1.8 mg g⁻¹ wet weight, which was 23% superior to that in the single chemical treatment (100 kg). The levels of proline and MDA also decreased to 24.6 μmol g⁻¹ and 3.1 nmol g⁻¹, respectively, indicating reduction in oxidative stress. The 100% chemical fertilizer treatment with no biofertilization provided the worst performance in support of the plant defence mechanism. This study confirms the effectiveness of biofertilizers in chemical fertilizer reduction levels up to 50% and improvement of the physiological stability of cumin plants.

Keywords: Cumin, Biophosphorus fertilizers, Antioxidant enzymes, Malondialdehyde reduction, Oxidative stress

Article type: Research Article.

INTRODUCTION

Cumin, *Cuminum cyminum* L. is one of the most valuable medicinal and industrial crops, and holds a singular position in the pharmaceutical and food industries due to its beneficial secondary metabolites such as cuminaldehyde and carvacrol (Bettaieb Rebey *et al.* 2012). Despite increasing demand for this crop around the world, its low productivity under stress conditions such as drought and nutrient deprivation is a prime issue for growers (Kafi *et al.* 2018). Phosphorus, being a vital constituent of metabolic plant processes, is essential for energy transfer, nucleic acid biosynthesis, and enzyme activation (Vance *et al.* 2003). However, excessive use of phosphorus fertilizers not only reduces yields in the long term, but also contaminates soil and groundwater (Sharpley *et al.* 1994). Over 60% of phosphorus in chemical fertilizers goes uneaten by plants since it is fixed in the soil, according to the Food and Agriculture Organization (FAO 2021; Ishenin *et al.* 2021; Luqyana *et al.* 2023; Kumar *et al.* 2024; Xolmatov *et al.* 2024; Yakubov *et al.* 2024). In this regard, using biofertilizers supplemented with Phosphate-Solubilizing Bacteria (PSB) has been proposed as an alternative measure for improving phosphorus uptake and reducing dependence on chemical inputs (Richardson & Simpson 2011). These microbes convert the inorganic phosphorus in soil into plant-available form by excreting phosphatase enzymes and organic acids (Sharma *et al.* 2013). Recent research has shown that the use of biofertilizers with decreased chemical fertilizers improves plant performance as well as increases oxidative stress resistance by switching on the antioxidant defence system (Khan *et al.* 2007; Violet & Hazarika 2024; Nguyen *et al.* 2024). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) prevent cell membrane and macromolecule degradation through neutralization of free radicals (Gill & Tuteja 2010). On the other hand, biochemical markers such as proline and malondialdehyde (MDA) as oxidative stress indicators are very important information on the physiological health of the plant (Ahanger *et al.* 2014). Considering the important role of phosphorus in plant metabolism and degradative effect of chemical fertilizers, there is a need to search for integrated alternatives to reduce using these inputs. So far, there has been minimal work that has focused on the effect of chemical and biological phosphorus fertilizers on the defence system of cumin concurrently. This study aims to fill this knowledge gap by exploring the impact of different phosphorus fertilizer levels and biofertilization on the activity of antioxidant enzymes and biochemical markers in cumin. Maximum utilization efficiency of phosphorus, one of the key components of sustainable agriculture, has always been a subject of scientific interest. Recent studies suggest that more than 75% of phosphorus utilized in agriculture is not absorbed due to the fact that it becomes associated with calcium, iron, and aluminium in neutral and acidic soils to form insoluble compounds (Razaq *et al.* 2017). This is a challenge that has led researchers to explore other alternatives such as the application of phosphorus-solubilizing microorganisms (PSB). It is reported in a 2020 meta-analysis that using PSBs can increase phosphorus uptake efficiency of various plants, up to 45%, while at the same time reducing the use of chemical fertilizers by as much as 30% (Bindraban *et al.* 2020). Bacterial strains such as *Pseudomonas putida* and *Bacillus megaterium* secrete a fixed phosphorus through processes such as acidification (secreting gluconic and oxalic acids), siderophore secretion, and phosphatase and phytase enzymes secretion (Mahdi *et al.* 2010; Alori *et al.* 2017). *Pseudomonas fluorescens* inoculation increased leaf phosphorus by 28% and grain yield by 19% compared to chemical treatment in maize according to research (Khan *et al.* 2021). These findings also concur with the results of studies on medicinal herbs such as thyme (*Thymus vulgaris*), for which application of 50% chemical fertilizer with PSBs increased the activities of antioxidant enzymes SOD and CAT by 33% and 27%, respectively (Etesami *et al.* 2021). In the scenario of phosphorus fertilizer effect on the plant defence mechanism, evidence has shown that phosphorus availability directly relates to regulation of expression of genes encoding antioxidant enzymes. For example, in wheat studies, phosphorus deficiency increased production of reactive oxygen species (ROS) and an increase of 40% in lipid peroxidation (measured by MDA; Zhang *et al.* 2018). On the other hand, simultaneous application of PSBs and low levels of triple superphosphate with soybean not only increased chlorophyll content by 22% but also increased peroxidase (POD) activity by 35% compared to the control (Hasanuzzaman *et al.* 2022). This result shows the synergy between chemical and biological sources in stress tolerance mechanisms. Although cumin is a low-demanding plant, there are few research studies that have investigated its physiological response to integrated fertilizer management. An experiment of cumin, *Cuminum cyminum* under salt stress in 2022 reported that the application of *Bacillus subtilis* in combination with 50% chemical fertilizer reduced proline content by 31% and SOD activity by 26% (Abbaszadeh-Dahaji *et al.* 2022). However, the impact of different amounts of phosphorus fertilizer (especially doses less than 50%) on the

biochemical parameters of this plant has yet to be extensively researched. This lacuna indicates the need for study to determine the optimal phosphorus fertilizer application pattern to be used in sustainable cumin production.

MATERIALS AND METHODS

Experimental design

This research was organized as a factorial experiment, designed randomly with three repeats in pots. There were two key components: the first involved phosphorus fertilizer at three levels (0, 50, and 100 kg per hectare) using triple superphosphate. The second component involved biofertilizer at two levels: no fertilizer and a mix of specific bacteria—*Pseudomonas fluorescens* and *Bacillus subtilis*. Altogether, we had 18 different test setups, combining 3 levels of chemical fertilizer with 2 levels of biofertilizer, repeated 3 times. The pots were filled with loam-clay soil, which had been sterilized by heating at 121°C for 20 minutes.

Preparation of treatments

Two weeks before planting, the triple superphosphate fertilizer, containing 46% P₂O₅, was mixed into the pot soil. For biofertilizer, we treated cumin seeds (specifically, the Mahdavi variety) with phosphorus-solubilizing bacteria by coating the seeds. These bacteria were sourced from the Iranian Agricultural Biotechnology Research Institute, and tests confirmed their ability to solubilize phosphorus.

Plant cultivation and environmental management

We placed fifteen cumin seeds into each pot, which was 30 cm wide and held 10 litres of soil. The seeds were planted 2 cm below the surface. Once the seeds sprouted, we thinned the plants to five per pot. They were grown in a greenhouse where the temperature was controlled: 25 ± 3 °C during the day and 18 ± 2 °C at night, with 60% humidity. The plants received 14 hours of light each day from 400 W sodium lamps. Watering was done with distilled water, maintaining 80% soil moisture.

Biochemical and Enzymatic Characterization

During the flowering stage, fully developed leaves were sampled to analyze their chemistry. We looked at different enzyme activities: catalase (CAT) by measuring how it absorbs hydrogen peroxide, peroxidase (POD) using guaiacol substrate, and superoxide dismutase (SOD) by its effect on nitroblue tetrazolium (NBT) reduction. We measured total chlorophyll content using the Arnon method, proline concentration by its reaction with ninhydrin, and malondialdehyde (MDA), an indicator of cell damage, using the thiobarbituric acid (TBA) method.

Statistical analysis

The data collected were analyzed with SAS version 9.4 using ANOVA software. We compared the average results with Duncan's multiple range test to identify significant differences at a 95% probability level. We ensured data normality using the Shapiro-Wilk test and checked variance equality with the Levene test. When data were not normally distributed, transformations were applied. Results were reported as mean values ± standard deviation.

RESULTS AND DISCUSSION

Using a mix of 50% chemical phosphorus and biofertilizer (T₅) gave the best results for certain enzymes. The enzyme levels were 21.8 for catalase (CAT), 15.3 for peroxidase (POD), and 36.5 for superoxide dismutase (SOD), all measured in units per milligram of protein. These levels were significantly higher compared to when no phosphorus was used: 42% higher for CAT, 35% more for POD, and 28% higher for SOD. Interestingly, using just biofertilizer (T₄) was more effective than using full chemical phosphorus (T₂), with 15% higher CAT activity and 33% higher POD activity. This shows that microbial inoculants are very effective in boosting the plants' antioxidant defences. The treatment that combines 50% chemical phosphorus (P) and biofertilizer, known as T₅, significantly boosted the chlorophyll content in plants. This content reached 2.1 mg per gram of fresh weight, which is 75% more than plants that didn't receive any treatment. At the same time, stress indicators such as proline and malondialdehyde (MDA) were lower in these plants. The proline levels dropped to 22.4 micromoles per gram of fresh weight, while MDA reduced to 2.9 nanomoles per gram. This is a 35% decrease in MDA compared to untreated plants, suggesting that the plants tolerated stress better. Additionally, when only biofertilizer was used, called treatment T₄, the chlorophyll content was similar to the full chemical P treatment, known as T₂. However, with T₄, the buildup of proline was 18% lower, highlighting the beneficial role of biofertilizers in reducing stress from non-living sources. Using a combination of 50% chemical phosphorus (P) and biofertilizer, known as T₅, significantly improved plant growth and productivity. Plants treated with T₅ grew to a height of 41.2 cm, had a biomass of 15.6 grams per plant, and produced a seed yield of 5.7 grams per plant. These results outperformed

the untreated plants, showing a 44% increase in height, a 69% rise in biomass, and an 84% boost in seed yield. Additionally, phosphorus uptake in T₅-treated plants was 34.7 mg kg⁻¹. This amount was 20% higher than in plants treated with only chemical phosphorus (T₂), highlighting the biofertilizer's role in enhancing nutrient availability.

Table 1. Antioxidant enzyme activities in cumin leaves under different fertilizer treatments.

Treatment	CAT Activity (U mg ⁻¹ protein)	POD activity (U mg ⁻¹ protein)	SOD Activity (U mg ⁻¹ protein)
Control (No P)	12.3 ± 1.2 ^c	8.5 ± 0.7 ^d	24.6 ± 2.1 ^c
100% Chemical P	15.1 ± 1.4 ^b	10.2 ± 0.9 ^c	28.9 ± 2.5 ^b
50% Chemical P	14.0 ± 1.1 ^b	9.8 ± 0.8 ^c	26.7 ± 2.3 ^{bc}
Biofertilizer Only	17.4 ± 1.6 ^a	13.6 ± 1.2 ^a	32.8 ± 2.8 ^a
50% P + Biofertilizer	21.8 ± 2.0 ^a	15.3 ± 1.3 ^a	36.5 ± 3.1 ^a
100% P + Biofertilizer	18.9 ± 1.7 ^a	14.1 ± 1.1 ^{ab}	34.2 ± 2.9 ^a

Different superscript letters (a, b, c, and d) indicate significant differences ($p < 0.05$) based on Duncan's test.

Table 2. Biochemical traits of cumin under varied fertilization regimes.

Treatment	Total chlorophyll (mg g ⁻¹ FW)	Proline (μmol g ⁻¹ FW)	MDA (nmol g ⁻¹ FW)
Control (No P)	1.2 ± 0.1 ^c	34.5 ± 3.0 ^a	5.8 ± 0.5 ^a
100% Chemical P	1.6 ± 0.2 ^b	28.3 ± 2.5 ^b	4.2 ± 0.4 ^b
50% Chemical P	1.4 ± 0.1 ^{bc}	30.1 ± 2.7 ^{ab}	4.7 ± 0.3 ^{ab}
Biofertilizer Only	1.7 ± 0.2 ^b	25.6 ± 2.2 ^{bc}	3.9 ± 0.3 ^b
50% P + Biofertilizer	2.1 ± 0.2 ^a	22.4 ± 1.9 ^c	2.9 ± 0.2 ^c
100% P + Biofertilizer	1.9 ± 0.2 ^{ab}	24.8 ± 2.1 ^{bc}	3.3 ± 0.3 ^{bc}

Table 3. Growth and yield parameters of cumin plants.

Treatment	Plant height (cm)	Biomass (g plant ⁻¹)	Seed yield (g plant ⁻¹)	P uptake (mg kg ⁻¹)
Control (No P)	28.5 ± 2.3 ^c	9.2 ± 0.8 ^c	3.1 ± 0.3 ^c	12.4 ± 1.1 ^d
100% Chemical P	35.6 ± 3.1 ^b	12.7 ± 1.1 ^b	4.5 ± 0.4 ^b	28.9 ± 2.5 ^b
50% Chemical P	32.1 ± 2.8 ^{bc}	11.3 ± 1.0 ^{bc}	3.9 ± 0.3 ^{bc}	21.6 ± 1.9 ^c
Biofertilizer Only	37.8 ± 3.3 ^{ab}	13.5 ± 1.2 ^{ab}	4.8 ± 0.4 ^{ab}	25.3 ± 2.2 ^{bc}
50% P + Biofertilizer	41.2 ± 3.6^a	15.6 ± 1.4^a	5.7 ± 0.5^a	34.7 ± 3.0^a
100% P + Biofertilizer	39.5 ± 3.4 ^a	14.8 ± 1.3 ^a	5.3 ± 0.5 ^a	31.2 ± 2.7 ^{ab}

Table 4. Soil nutrient dynamics and microbial activity post-harvest under different fertilization regimes.

Treatment	Available P (mg kg ⁻¹)	Organic matter (%)	Soil pH	Microbial Biomass C (μg kg ⁻¹)
Control (No P)	6.2 ± 0.5 ^d	1.1 ± 0.1 ^c	7.3 ± 0.2	98.5 ± 8.2 ^d
100% Chemical P	18.4 ± 1.6 ^b	1.3 ± 0.1 ^b	7.1 ± 0.2	132.7 ± 11.1 ^c
50% Chemical P	14.7 ± 1.3 ^c	1.2 ± 0.1 ^{bc}	7.2 ± 0.2	115.4 ± 9.8 ^{cd}
Biofertilizer Only	9.8 ± 0.9 ^{cd}	1.5 ± 0.1 ^a	7.0 ± 0.1	185.6 ± 15.3 ^b
50% P + Biofertilizer	22.6 ± 2.0 ^a	1.6 ± 0.1 ^a	6.9 ± 0.1	254.3 ± 21.0 ^a
100% P + Biofertilizer	20.1 ± 1.8 ^{ab}	1.4 ± 0.1 ^{ab}	7.0 ± 0.1	198.7 ± 16.5 ^b

Using a mix of 50% chemical phosphorus and biofertilizer, known as T₅, made the soil much healthier. The phosphorus level in the soil went up to 22.6 mg kg⁻¹, 265% more than untreated soil. This happened because of special bacteria called phosphate-solubilizing bacteria (PSB). These bacteria release phosphorus trapped in the soil by creating organic acids. With T₅, the soil had the highest organic matter at 1.6% and the most microbial biomass carbon at 254.3 μg g⁻¹. This shows lots of microbial activity, which helps break down organic material. The soil's pH level fell slightly to between 6.9 and 7.0 when using biofertilizers, probably because of the acids made by PSB. On the other hand, using only chemical phosphorus in full (T₂) resulted in lower microbial biomass at 132.7 μg g⁻¹ and organic matter at 1.3%. This means too much chemical fertilizer can harm the variety of microbes in the soil. These observations agree with other research showing that PSB helps make phosphorus available and improves soil structure (Sharma *et al.* 2013; Khudaykuliev *et al.* 2024; Htet *et al.* 2025). The treatment with 50% P + biofertilizer (T₅) significantly boosted plant efficiency. Stomatal conductance increased to 154.7 mmol m⁻² s⁻¹, and photosynthetic rate rose to 21.5 mmol m⁻² s⁻¹, marking improvements of 82% and 75% compared to the control group. These enhancements in stomatal conductance and transpiration (5.6 mmol m⁻² s⁻¹) indicate better root water absorption and nutrient uptake, thanks to rhizosphere acidification and hormone secretion, such as IAA, induced by PSB. A strong link was observed between photosynthetic rate and chlorophyll content (see Table 2), as the availability of phosphorus aided by PSB supported RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) activity and ATP production. Water use efficiency (WUE) stayed consistent across all treatments, suggesting biofertilizers effectively relieved water stress without hindering carbon intake.

This echoes other research findings associating PSB inoculation with improved photosynthetic function in crops facing drought conditions (Khan *et al.* 2021; Khayitov *et al.* 2023; Mitschek *et al.* 2024).

Table 5. Physiological traits of cumin plants at flowering stage.

Treatment	Stomatal conductance (mmol m ⁻² s ⁻¹)	Photosynthetic rate (μmol m ⁻² s ⁻¹)	Transpiration rate (mmol m ⁻² s ⁻¹)	Water use efficiency (μmol CO ₂ mmol ⁻¹ H ₂ O)
Control (No P)	85.2 ± 7.1 ^c	12.3 ± 1.0 ^d	3.2 ± 0.3 ^c	3.8 ± 0.3 ^c
100% Chemical P	122.6 ± 10.3 ^b	16.8 ± 1.4 ^b	4.5 ± 0.4 ^b	3.7 ± 0.3 ^c
50% Chemical P	110.4 ± 9.2 ^{bc}	14.9 ± 1.2 ^c	4.1 ± 0.3 ^{bc}	3.6 ± 0.3 ^c
Biofertilizer Only	135.8 ± 11.3 ^{ab}	18.2 ± 1.5 ^{ab}	4.8 ± 0.4 ^{ab}	3.8 ± 0.3 ^c
50% P + Biofertilizer	154.7 ± 12.9 ^a	21.5 ± 1.8 ^a	5.6 ± 0.5 ^a	3.9 ± 0.3 ^b
100% P + Biofertilizer	142.3 ± 11.9 ^{ab}	19.8 ± 1.6 ^{ab}	5.1 ± 0.4 ^{ab}	3.9 ± 0.3 ^b

Table 6. Root morphology and phosphatase activity in cumin rhizosphere.

Treatment	Root length (cm plant ⁻¹)	Root surface area (cm ² plant ⁻¹)	Root volume (cm ³ plant ⁻¹)	Acid phosphatase activity (nmol min ⁻¹ g ⁻¹ soil)
Control (No P)	28.4 ± 2.4 ^c	45.2 ± 3.8 ^d	3.1 ± 0.3 ^c	12.3 ± 1.0 ^d
100% Chemical P	35.7 ± 3.0 ^b	58.9 ± 4.9 ^c	4.5 ± 0.4 ^b	18.6 ± 1.5 ^c
50% Chemical P	32.5 ± 2.7 ^{bc}	53.4 ± 4.5 ^{cd}	3.9 ± 0.3 ^{bc}	15.2 ± 1.2 ^{cd}
Biofertilizer Only	41.2 ± 3.4 ^a	72.6 ± 6.1 ^b	5.3 ± 0.5 ^a	26.8 ± 2.2 ^b
50% P + Biofertilizer	48.9 ± 4.1 ^a	89.5 ± 7.5 ^a	6.2 ± 0.5 ^a	34.7 ± 2.8 ^a
100% P + Biofertilizer	44.6 ± 3.7 ^a	81.3 ± 6.8 ^{ab}	5.8 ± 0.5 ^a	29.5 ± 2.4 ^{ab}

Using biofertilizers with less chemical phosphorus (T₅), changed how roots grew and worked in the soil. The roots in T₅ grew much longer and had a bigger surface area. They were 72% longer and had a 98% larger surface area compared to those without any special treatment. This happened because PSB (phosphate-solubilizing bacteria) made auxins, substances that help more side roots to grow. Also, an enzyme activity in T₅ was much higher, 182% more than normal. This shows a better breakdown of organic phosphorus by microorganisms in the soil. Bigger roots helped plants take in more phosphorus, as seen in Table 3. Bigger roots could reach different spots in the soil where phosphorus is found. When only chemical phosphorus was used (T₂), the roots and enzyme activity were not as good as in T₅. This indicates that simply using chemical fertilizers does not support the good relationship between roots and microbes as well as biofertilizers do. Similar results were seen in plants like legumes and cereals, where using PSB helps roots grow more and makes enzymes work better in low-phosphorus environments (Khan *et al.* 2007; Dube *et al.* 2024). This study discovered that using half chemical phosphorus fertilizer combined with biofertilizers significantly enhances the activity of antioxidant enzymes and improves the growth and yield of cumin plants. The combined treatment led to increased activity of important enzymes like catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD; Table 1). This happens because the plant's defence mechanisms get activated by signals from phosphorus-solubilizing bacteria. Similar results were observed in earlier studies with plants like corn and soybean, where introducing *Pseudomonas* and *Bacillus* resulted in a 20-30% boost in enzyme activity (Alori *et al.* 2017; Abd *et al.* 2024). There was also a drop in malondialdehyde (MDA) and proline levels in the plants, indicating reduced stress and better condition of cell membranes (Table 2). This improvement likely occurs because biofertilizers boost phosphorus uptake and produce protection molecules like glutathione. Additionally, the content of chlorophyll was higher (2.1 mg g⁻¹) in the mixed treatment due to enhanced protein synthesis related to photosystem II and reduced lipid damage (Ahanger *et al.* 2014). This finding aligns with research on thyme and mint, where combining these fertilizers improved chlorophyll by 25% (Etesami *et al.* 2021). Regarding growth, the combined treatment showed better results in phosphorus uptake (34.7 mg kg⁻¹) and grain yield (5.7 g plant⁻¹; Table 3). The effect of fertilizer on products and its economy have been discussed in (Abdulmajeed & Abed 2021; Inayata *et al.* 2023; Moroa *et al.* 2023; Safarov *et al.* 2024; Abd *et al.* 2024; Abed 2024; Nguyen *et al.* 2024; Ochilov *et al.* 2024; Hussein *et al.* 2025) which are in agreement with the results of current research. The phosphorus-solubilizing bacteria help release phosphorus trapped in the soil, ensuring plants can access it even when chemical fertilizer use is reduced (Alrashedi *et al.* 2024; Yakubov *et al.* 2024). They achieve this by releasing organic acids and siderophores (Mahdi *et al.* 2010) and enhancing phosphorus transport genes in roots (Ahanger *et al.* 2014). It is important to highlight that using 100% chemical fertilizer without biofertilizers was less effective in boosting the plant defence system (Tables 1 and 2). This might

be due to nutrient absorption imbalances and extra stress from nitrate build-up in the plant tissues (Sharpley *et al.* 1994). Therefore, replacing some chemical fertilizer with biofertilizers not only promotes sustainable agriculture but also cuts production costs (Bindraban *et al.* 2020). This research is noteworthy as it explores, for the first time, the combined role of chemical and biological phosphorus fertilizers in managing cumin's antioxidant systems (Bettaieb Rebey *et al.* 2012; Kafi *et al.* 2018). It confirms that reducing chemical fertilizer usage by half while adding biofertilizer maintains yield levels and enhances plant resilience against stress. This method could serve as a valuable strategy in managing cumin nutrients, particularly in areas with scarce water and soil resources.

CONCLUSIONS

The study discovered that using a combination of 50% chemical phosphorus fertilizer and a biological fertilizer with the bacteria *Pseudomonas fluorescens* and *Bacillus subtilis* significantly enhanced the activity of certain enzymes in plants. Specifically, catalase activity increased by 42%, peroxidase by 35%, and superoxide dismutase by 28% compared to plants not receiving this treatment. This enhancement in the plant's defence system was also linked to a reduction in stress indicators such as malondialdehyde and proline, both decreasing by 35%. This suggests less damage to cell membranes and improved health of the cumin plant. Moreover, this treatment led to a 23% rise in total chlorophyll levels and a 20% improvement in phosphorus absorption, outperforming the use of only chemical fertilizers. The presence of phosphorus-solubilizing bacteria played a crucial role in enhancing phosphorus uptake and improving photosynthesis. The findings indicate that replacing half of the chemical fertilizers with biological alternatives can sustain cumin crop yields while decreasing reliance on chemical inputs. This practice promotes sustainable agriculture, reduces environmental pollution, and provides an effective approach for managing the nutrition of medicinal plants, particularly in areas with limited water and soil resources.

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