

## **Influence of different classes of agrochemicals on the growth and viability of microorganisms in biofertilizers**

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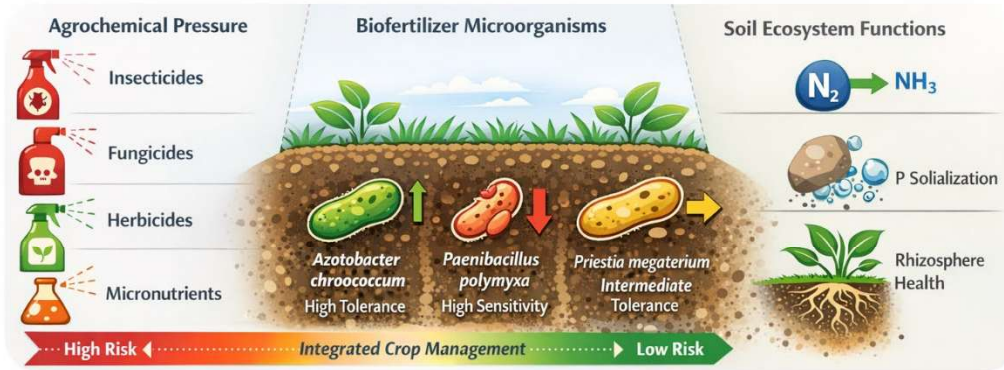
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**Abstract.** The compatibility of biofertilizers with conventional agrochemicals is a critical but underexplored factor influencing integrated crop management. While biofertilizers offer sustainable alternatives to chemical inputs, their simultaneous application with pesticides and micronutrient formulations may compromise microbial viability and functionality. This study systematically evaluated the in vitro compatibility of commonly used agrochemicals with microbial strains constituting the biofertilizers N-Fixera (*Azotobacter chroococcum* MDC 6111) and PhosRhiza (*Paenibacillus polymyxa* MDC 280 and *Priestia megaterium* MDC 2124). Microbial responses were assessed using a disc diffusion assay on Petri dishes, with growth stimulation or inhibition scored on a five-point scale. Agrochemicals were classified as insecticides, herbicides, fungicides, or micronutrient formulations. Data were integrated via heatmaps and class-level averaging to reveal general compatibility patterns. Results demonstrated pronounced strain-specific and chemical class-dependent effects. Insecticides and fungicides exerted the strongest selective pressure, markedly inhibiting spore-forming Gram-positive bacteria, whereas *A. chroococcum* exhibited higher tolerance and occasional stimulation. Herbicides were largely neutral or weakly stimulatory across all strains, indicating minimal direct microbial toxicity. Micronutrient formulations showed a narrow safety margin, with complex mixtures strongly inhibiting nitrogen-fixing bacteria. These findings indicate that compatibility cannot be generalized at the product level and must be evaluated at the strain level. The proposed visualization-based framework offers a practical tool for optimizing the combined use of biological fertilizers and agrochemicals, enhancing the reliability, sustainability, and efficiency of integrated crop management strategies.

**Key words:** biofertilizers, agrochemical compatibility, nitrogen-fixing bacteria, phosphate-solubilizing bacteria, integrated crop management, microbial tolerance.

**Graphical abstract.** Balancing agrochemicals with biofertilizers is key to sustaining soil microbial functions.



## INTRODUCTION

The paradigm of agricultural intensification that dominated the second half of the twentieth century relied heavily on the extensive use of mineral fertilizers and chemical crop protection agents. Although this approach ensured short-term yield increases, it also resulted in progressive soil degradation, biodiversity loss, disruption of biogeochemical cycles, and reduced ecosystem resilience. In response, modern agroecology emphasizes the integration of biologically based inputs aimed at restoring soil functionality and reducing the ecological footprint of agricultural systems (Vessey, 2003; Bardgett & van der Putten, 2014).

Biofertilizers (biopreparations) represent a key component of this transition. These products consist of living microorganisms or their metabolites that enhance nutrient availability, stimulate plant growth, suppress phytopathogens, and improve plant tolerance to abiotic stress (Bhattacharyya & Jha, 2012; Harutyunyan et al., 2025b). Their effects are mediated through mechanisms such as biological nitrogen fixation, solubilization of phosphorus and micronutrients, production of phytohormones and siderophores, and modulation of plant immune responses (Vessey, 2003; Glick, 2012; Bagiyan et al., 2024).

Microbial taxa commonly included in biofertilizers, including *Azotobacter* spp., *Azospirillum* spp., *Paenibacillus polymyxa*, *Priestia megaterium*, and *Bacillus* spp., etc. play essential roles in maintaining soil fertility and plant productivity. Nitrogen-fixing bacteria contribute biologically fixed nitrogen, reducing dependence on synthetic fertilizers (Khan et al., 2007; Harutyunyan et al., 2024), while phosphate-solubilizing microorganisms convert insoluble phosphorus into plant-available forms (Rodríguez & Fraga, 1999). In addition, plant growth-promoting rhizobacteria (PGPR) enhance plant performance through phytohormone production and improved nutrient uptake (Lugtenberg & Kamilova, 2009). These microbial groups also contribute to the stability and resilience of soil microbial communities, which is a critical determinant of agroecosystem functioning under environmental stress (Wittebolle et al., 2009; Khachatryan et al., 2023; Goginyan et al., 2025; Harutyunyan et al., 2025a).

Despite the ecological benefits of biological fertilizers, their performance in agricultural systems is strongly influenced by the prevailing chemical environment. Modern crop production relies heavily on herbicides, insecticides, fungicides, and micronutrient formulations, often applied repeatedly and in combination. A growing body of evidence indicates that these agrochemicals exert significant non-target effects on soil microbial communities, affecting their diversity, metabolic activity, and ecological interactions (Lo, 2010; Imfeld & Vuilleumier, 2012).

From a microbial ecology perspective, agrochemicals act as selective stressors capable of reshaping community composition and functional potential. Prolonged exposure may reduce microbial diversity, alter community structure, and impair ecosystem functioning, thereby limiting the establishment and persistence of introduced microbial inoculants (Wittebolle et al., 2009; Pooja & Sagar, 2025).

Herbicides represent the most widely used class of agrochemicals. Although primarily designed to target plant-specific metabolic pathways, they may also affect soil microorganisms either directly or indirectly through modifications of the rhizosphere environment. For example, glyphosate has been shown to alter microbial community composition and metabolic processes in soil (Balota et al., 1998; Ratcliff et al., 2006), while synthetic auxins can influence microbial dynamics by modifying root exudation patterns (Grossmann, 2010; Schulz & Segobye, 2016). Repeated herbicide applications may also suppress enzymatic activity, nitrogen fixation, and phosphate solubilization, ultimately affecting the functional efficiency of microbial inoculants (Imfeld & Vuilleumier, 2012; Sushma et al., 2024).

Insecticides, including pyrethroids, neonicotinoids, and avermectins, are primarily neurotoxic to arthropods but may also influence soil microbial processes. Their persistence in soil and interaction with organic matter can lead to prolonged exposure of microbial communities (Cycoń & Piotrowska-Seget, 2016). Experimental studies have demonstrated that insecticides can affect microbial respiration, nitrogen cycling, and biomass, with outcomes depending on compound type, concentration, and exposure duration (Bai et al., 2023).

Fungicides are specifically designed to inhibit fungal growth and therefore often exert pronounced effects on non-target microbial communities. Alterations in fungal populations may have cascading consequences for bacterial communities due to their ecological interdependence (Baćmaga et al., 2016). Repeated fungicide application has been associated with reduced microbial activity and functional diversity, potentially affecting the stability of microbial consortia used in biological fertilizers.

Micronutrient formulations, including copper, zinc, and iron chelates, play an important role in plant nutrition but may exert dual effects on microorganisms. While essential at low concentrations, elevated levels can induce oxidative stress and inhibit microbial activity (Giller et al., 1998). This highlights the importance of precise dosage and compatibility assessment when micronutrients are applied alongside microbial inoculants (Mikaelyan et al., 2025).

Collectively, the available evidence highlights that agrochemical-microbe interactions are highly complex, context-dependent, and often underestimated in agricultural practice. While chemical input remains indispensable for pest and disease control, their non-target effects on beneficial microorganisms necessitate a more integrated and ecologically informed management strategy.

Despite increasing adoption of biofertilizers, a critical knowledge gap remains regarding their compatibility with commonly used agrochemicals. This study aims to systematically evaluate the effects of 17 agrochemicals on the growth, viability, and functional activity of microorganisms in two commercial biofertilizers, N-Fixera and PhosRhiza.

We hypothesize that the compatibility between agrochemical and microbial biofertilizers varies significantly depending on agrochemical class and microbial strain, due to differences in their physiological and structural characteristics.

The specific objectives are: (i) to assess the impact of insecticides, herbicides, fungicides, and microelements on selected microbial strains (*A. chroococcum*, *P. polymyxa*, *P. megaterium*); (ii) to determine stimulatory or inhibitory interactions and identify compatible and antagonistic combinations; (iii) to provide empirical evidence for optimizing the integrated use of biological fertilizers and chemical crop protection agents; and (iv) to support the development of guidelines for safe and effective application of biopreparations in agroecosystems exposed to routine agrochemical inputs. By achieving these objectives, this study will enhance understanding of agrochemical-microbe interactions, facilitate the rational integration of biological fertilizers into sustainable agriculture, and contribute to evidence-based strategies for mitigating the non-target effects of chemical inputs while maintaining soil health and crop productivity.

## MATERIALS AND METHODS

### Microbial biofertilizers and bacterial strains

Two liquid microbial biofertilizers (potential producer “Biona” LLC, Russia) were used in the experiments. Both products were developed at the Scientific and Production Center “Armbiotechnology” NAS RA and sequentially evaluated under laboratory and field conditions prior to their use in the present study.

N-Fixera is a nitrogen-fixing biofertilizer based on *Azotobacter chroococcum* (Beijerinck, 1901) MDC<sup>1</sup> 6111, containing viable cells at a concentration of  $10^7$ – $10^9$  CFU L<sup>-1</sup>. The strain exhibits high atmospheric nitrogen (N) fixation capacity (up to 13.3 mg N per g of fermented carbon source) and produces a range of biologically active metabolites, including amino acids, organic acids, and polysaccharides. The biofertilizer enhances soil fertility, improves plant nutrition, and increases plant tolerance to abiotic stress and bacterial pathogens. The biofertilizer is effective in soils with pH ranging from 6.0 to 8.0.

PhosRhiza is a dual-component biofertilizer comprising *Paenibacillus polymyxa* (Prazmowski, 1880; Ash et al., 1993) MDC 280 and *Priestia megaterium* (de Bary, 1884; Gupta et al., 2020; Biedendieck et al., 2021) MDC 2124, each at  $1.0 \times 10^7$ – $1.0 \times 10^9$  CFU L<sup>-1</sup>. The consortium combines phosphate-solubilizing activity (up to 280 mg L<sup>-1</sup> Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>) with N-fixation and rhizosphere colonization capacity, contributing to improved nutrient availability and plant growth. The biofertilizer is effective in soils with pH values between 5.5 and 8.5.

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<sup>1</sup> MDC, the official acronym of the National Culture Collection of Non-Pathogenic Microorganisms.

All bacterial strains used in the formulations (*A. chroococcum*, *P. polymyxa*, and *P. megaterium*) were obtained from the Microbial Depository Center of the Scientific and Production Center “Armbiotechnology” NAS RA, studied in detail and maintained under standard laboratory conditions.

Functionally, *A. chroococcum* MDC 6111 acts as a free-living diazotroph with additional antagonistic activity against phytopathogens, contributing to both nitrogen input and plant protection. *P. polymyxa* MDC 280 exhibits dual functionality, combining nitrogen fixation with strong phosphate-solubilizing activity and enzymatic capabilities typical of plant growth-promoting rhizobacteria (PGPR). *P. megaterium* MDC 2124 is characterized by efficient rhizosphere colonization and the ability to mobilize mineral nutrients (P, Ca, Fe, Mg) through organic acid production, as well as high environmental resilience due to spore formation.

### **Characterization of agrochemicals**

For clarity and comparability, the agrochemicals used in this study are classified in Table S1 according to their functional class, active ingredients, primary mechanism of action (MoA) and key characteristics. Agrochemicals were applied at concentrations corresponding to typical field-equivalent working solutions and recalculated to active ingredient concentrations. Dose classification reflects expected biological relevance for microbial systems, e.g. sublethal, field-equivalent, or potentially inhibitory.

### **Microbial strains and inoculum preparation**

The compatibility of selected agrochemicals with microbial strains was evaluated *in vitro* using the disc diffusion method on Petri plates. The study included agrochemical formulations encompassing insecticides, herbicides, fungicides, and micronutrients. Each treatment was tested in triplicate, and all experiments were repeated twice to ensure reproducibility (n = 6).

*A. chroococcum* MDC 6111: cultured on Ashby agar (Ettema, 2000) at 28 °C for 48 h. *P. polymyxa* MDC 280 and *P. megaterium* MDC 2124 cultured on nutrient agar (Harrigan & McCance, 1966; Saghyan et al., 2021) at 30 °C for 48 h. For each assay, fresh bacterial suspensions were prepared by harvesting mid-logarithmic phase cultures and adjusting the density to approximately  $10^8$  CFU L<sup>-1</sup>, ensuring uniform lawn growth on agar surfaces.

### **Disc diffusion assay**

*Plate preparation.* Sterile Petri plates (90 mm) were filled with 20 L of appropriate agar medium (Ashby for *A. chroococcum*, nutrient agar for *P. polymyxa* and *P. megaterium*). Agar surfaces were evenly inoculated with microbial suspensions using sterile swabs to create a uniform bacterial lawn.

*Disc preparation and agrochemical application.* Sterile filter paper discs (6 mm diameter) were impregnated with 10 µL of agrochemical solution, prepared at field-equivalent concentrations (Table S1) and recommended by the manufacturer. Three replicate discs were placed equidistantly on each inoculated plate.

*Incubation conditions.* Plates were incubated at 28–30 °C for 48–72 hours, depending on the growth rate of the strain, under aerobic conditions.

*Assessment of microbial response.* The effects of agrochemicals on microbial growth were assessed using a semi-quantitative scoring system based on the agar disk diffusion

method. This approach is widely applied as a screening technique in microbiology and is inherently semi-quantitative, as it evaluates growth inhibition based on the size and characteristics of inhibition zones rather than exact concentration-response relationships (Balouiri et al., 2016).

In standard protocols, inhibition zones are typically interpreted using categorical classifications (e.g., susceptible, intermediate, resistant), reflecting the qualitative nature of the method (Tenover, 2009). In this study, this concept was extended into a 5-point scale (Table 1) to allow finer discrimination between levels of stimulation and inhibition.

The scoring system was calibrated based on visual assessment of inhibition zone intensity and growth characteristics, consistent with semi-quantitative interpretations of disk diffusion assays (van de Klundert et al., 1986).

**Table 1.** Semi-quantitative scoring scale for microbial growth response to agrochemical exposure

Score	Description
1	No visible effect (neutral)
2	Slight stimulation or inhibition
3	Moderate stimulation or inhibition
4	Strong stimulation or inhibition
5	Very strong stimulation or inhibition / complete growth suppression

*Comments:* Stimulation zones: bacterial growth extending beyond the control lawn or increased colony density near the disc. Inhibition zones: clear or partially cleared areas around the disc where microbial growth was reduced or absent. Negative control (NC): plates with discs treated with sterile water or carrier solution only. Solvent control (SC): plates treated with the corresponding carrier or adjuvant solution without the active ingredient, to account for non-specific effects.

### Calculation of mean microbial response scores

For each microorganism, responses to individual agrochemicals were evaluated using a 5-point semi-quantitative scoring system based on three independent replicates.

The mean score for each agrochemical was calculated as:

$$\bar{X}_{ij} = \frac{1}{n} \sum_{k=1}^n X_{ijk} \quad (1)$$

where  $X_{ijk}$  – score for microorganism  $i$ , agrochemical  $j$ , replicate  $k$ ;  $n = 3$  – number of replicates.

The standard deviation (SD) was calculated as:

$$SD_{ij} = \sqrt{\frac{1}{n-1} \sum_{k=1}^n (X_{ijk} - \bar{X}_{ij})^2} \quad (2)$$

Class-level mean responses were then calculated as:

$$\bar{X}_i^{class} = \frac{1}{m} \sum_{j=1}^m \bar{X}_{ij} \quad (3)$$

where  $m$  – number of agrochemicals within a given class.

For visualization purposes (heatmap), the original 5-point scoring scale (1–5) was transformed into a symmetric scale ranging from –5 to +5. In this transformation, inhibitory effects were assigned negative values (–1 to –5), stimulatory effects were assigned positive values (+1 to +5), and neutral responses were centered around zero. This transformation was applied solely for graphical representation to improve interpretability and does not affect the underlying semi-quantitative assessment.

### Statistics

Due to the semi-quantitative nature of the scoring system and the limited number of replicates ( $n=3$ ), the data were primarily analyzed using descriptive statistics (mean  $\pm$  standard deviation). The results are presented as class-level averages to identify general trends in microbial responses to different agrochemical groups. Statistical comparisons were interpreted cautiously, and emphasis was placed on biologically meaningful differences rather than strict inferential testing.

## RESULTS AND DISCUSSION

### Strain-specific compatibility of agrochemicals with biofertilizer microorganisms

In vitro screening of 17 commercially available agrochemicals revealed pronounced strain-specific and formulation-dependent effects on microorganisms constituting the biofertilizers N-Fixera (*A. chroococcum* MDC 6111) and PhosRhiza (*P. polymyxa* MDC 280 and *P. megaterium* MDC 2124). Microbial responses spanned a broad continuum, ranging from growth stimulation to strong inhibition, underscoring the complexity of agrochemical–microbe interactions.

Importantly, these results demonstrate that compatibility cannot be inferred at the product or even genus level. Instead, individual strains within commercial biofertilizer formulations exhibit distinct tolerance thresholds, reflecting differences in cell envelope architecture, metabolic flexibility, and stress-response capacity. This finding aligns with earlier reports emphasizing strain-level heterogeneity in microbial sensitivity to xenobiotics but extends them by providing a systematic, cross-class comparison under standardized conditions (Afata et al., 2024; Hu et al., 2026).

### Agrochemical class-dependent response patterns

To identify generalized trends, microbial responses were integrated by agrochemical class (Table 2). Fungicides and insecticides exerted the strongest biological effects, whereas herbicides were largely compatible across all tested strains. Microelement formulations occupied an intermediate position, displaying a narrow margin between nutritional benefit and toxicity.

**Table 2.** Mean microbial responses (mean  $\pm$  SD) to agrochemical classes

Microorganism	Insecticides	Herbicides	Fungicides	Microelements
<i>A. chroococcum</i> MDC 6111	3.0 $\pm$ 0.7	2.0 $\pm$ 0.5	3.3 $\pm$ 0.8	1.5 $\pm$ 0.2
<i>P. polymyxa</i> MDC 280	2.0 $\pm$ 0.6	2.3 $\pm$ 0.5	1.7 $\pm$ 0.7	1.8 $\pm$ 0.3
<i>P. megaterium</i> MDC 2124	2.3 $\pm$ 0.5	2.1 $\pm$ 0.4	1.5 $\pm$ 0.6	2.0 $\pm$ 0.2

*Notes:* Values are expressed as mean  $\pm$  SD ( $n=3$  replicates per agrochemical). Individual agrochemical responses were first averaged across replicates, and class-level means were calculated as arithmetic averages of all agrochemicals within each group without weighting. Scores range from 1 (no effect) to 5 (complete inhibition).

Among the tested microorganisms, *A. chroococcum* MDC 6111 consistently exhibited the highest tolerance and, in several cases, moderate growth stimulation. In contrast, the Gram-positive, spore-forming strains *P. polymyxa* and *P. megaterium* were markedly more sensitive, particularly to insecticides and fungicides. This pattern

strongly suggests that Gram status and associated cell envelope structures play a central role in determining agrochemical susceptibility.

Mean response scores confirmed that fungicides represent the most biologically active agrochemical class, followed by insecticides, whereas herbicides exerted minimal direct toxicity (Chinen, 2023; Riedo et al., 2025). This pattern is consistent with the established modes of action of these compounds and their documented non-target effects on soil microbiota. Fungicides are specifically designed to inhibit or eliminate fungal cells, and therefore tend to exert pronounced effects on microbial systems. Insecticides primarily target insect nervous systems but may also induce secondary effects on microbial communities. In contrast, herbicides are intended to disrupt plant physiological processes and generally demonstrate lower direct toxicity toward microbial cells (Meena et al., 2020; Jeyaseelan et al., 2024; Wang et al., 2025).

### Direction and intensity of microbial responses

Directional analysis further refined these class-level trends (Table 3). *A. chroococcum* displayed mixed or stimulatory responses to insecticides and herbicides and moderate stimulation in response to fungicides, while complex microelement formulations caused pronounced inhibition. Such low-dose stimulation is indicative of hormetic effects, where sublethal stress activates protective metabolic and antioxidant pathways.

**Table 3.** Direction and intensity of microbial responses to agrochemical classes

Microorganism	Insecticides	Herbicides	Fungicides	Microelements
<i>A. chroococcum</i> MDC 6111	± / ↑	± / ↑	↑	↓↓
<i>P. polymyxa</i> MDC 280	↓↓	± / ↑	↓	↓
<i>P. megaterium</i> MDC 2124	↓	±	↓↓	±

Notes: ↑ = stimulation; ↓ = inhibition; ± = no significant effect; arrow number indicates intensity.

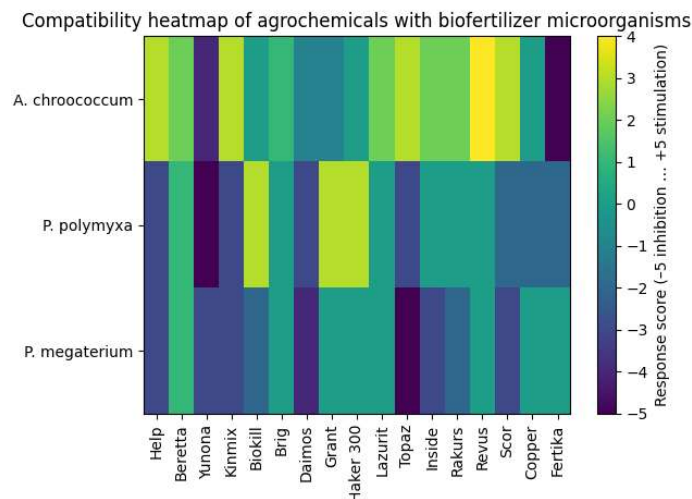
By contrast, *P. polymyxa* was strongly inhibited by insecticides and moderately inhibited by fungicides, while herbicides and microelements induced weak or neutral effects. *P. megaterium* showed intermediate sensitivity, with inhibition primarily associated with insecticides and fungicides. The heatmap visualization (Fig. 1) clearly illustrates that insecticides and fungicides impose the strongest selective pressure on microbial inoculants, whereas herbicides generally exhibit low direct microbial toxicity.

### Microbial responses to individual agrochemicals

Detailed semi-quantitative responses of the tested microorganisms to individual agrochemicals are presented in Table S2. The data reveals pronounced variability in both the intensity and direction of microbial responses depending on the agrochemical type and microbial species.

Among insecticides, the strongest inhibitory effects were observed for Yunona, particularly against *P. polymyxa* ( $5.0 \pm 0.0$ ) and *A. chroococcum* ( $4.0 \pm 0.7$ ), indicating high sensitivity of these strains to avermectin-based compounds. In contrast, Beretta exhibited minimal effects, especially on *P. polymyxa* and *P. megaterium* ( $1.0 \pm 0.0$ ), suggesting relatively low toxicity under the tested conditions. Biokill demonstrated species-specific responses, with neutral effects on *A. chroococcum* but moderate stimulation of *P. polymyxa*.

Herbicides generally showed weak or negligible effects on microbial growth, confirming their lower direct toxicity. However, Daimos induced clear inhibitory responses, particularly in *P. megaterium* ( $4.0 \pm 0.7$ ), indicating selective sensitivity to synthetic auxin-type herbicides. Other herbicides, including Brig and Lazurit, exhibited minimal or neutral effects across most strains.



**Figure 1.** Compatibility heatmap of different agrochemicals with biofertilizer microorganisms (*A. chroococcum*, *P. polymyxa*, *P. megaterium*). The color scale represents transformed semi-quantitative response scores ranging from  $-5$  (dark purple, strong growth inhibition) to  $+5$  (yellow, strong growth stimulation), derived from the original 5-point scoring system (1–5). Inhibitory effects were assigned negative values, stimulatory effects positive values, and neutral responses were centered around zero. Intermediate colors indicate moderate inhibitory (blue to teal) or weak stimulatory effects (green), while values close to zero correspond to negligible or no detectable effect on microbial growth.

Fungicides displayed the highest variability and overall strongest inhibitory potential. Topaz caused severe inhibition in *P. megaterium* ( $5.0 \pm 0.0$ ), while Inside and Scor also induced moderate inhibitory effects. In contrast, Revus showed a stimulatory effect on *A. chroococcum* ( $4.0 \pm 0.7$ ) but no detectable impact on the other strains, highlighting compound-specific and species-dependent responses.

Microelement formulations demonstrated contrasting effects. Copper chelate exhibited mild inhibitory activity toward *P. polymyxa*, whereas Fertika caused strong inhibition in *A. chroococcum* ( $5.0 \pm 0.0$ ), indicating potential toxicity of complex nutrient formulations at the tested concentrations.

Comparative grouping analysis (Table S2) further confirms that differences between microorganisms were often substantial ( $\geq 1$  score unit), reflecting distinct physiological sensitivities. Overall, *P. polymyxa* and *P. megaterium* tended to be more sensitive to inhibitory effects, whereas *A. chroococcum* more frequently exhibited neutral or stimulatory responses, suggesting higher adaptive tolerance.

### Integrated assessment of agrochemical classes

A consolidated overview of agrochemical effects (Table 4) confirms that fungicides pose the greatest risk to biofertilizer microorganisms, followed by insecticides. Herbicides demonstrated the highest overall compatibility, supporting their inclusion in integrated crop management systems when applied at recommended doses.

**Table 4.** Overall effects of agrochemical classes on microbial strains used in biological fertilizers

Agrochemical class	Microbial formulation of biofertilizers			General trend
	N-Fixera <i>A. chroococcum</i> MDC 6111	PhosRhiza <i>P. polymyxa</i> MDC 280	<i>P. megaterium</i> MDC 2124	
Insecticides (Lal & Shivaji, 1984; Sroczyńska et al., 2024)	Weak-moderate stimulation to inhibition	Moderate-strong inhibition	Moderate inhibition	High strain-specific variability
Herbicides (Devine et al., 1993; Jeyaseelan et al., 2024)	Weak inhibition to stimulation	Neutral to moderate stimulation	Mostly neutral	Generally low toxicity
Fungicides (Xu et al., 2024; Wang et al., 2025)	Moderate stimulation or tolerance	Moderate inhibition	Moderate-strong inhibition	Most biologically active class
Microelements (Chen et al., 2024)	Strong inhibition (complex formulations)	Weak-moderate inhibition	Neutral	Narrow safety margin

Microelement formulations emerged as an underappreciated risk factor. While often regarded as benign or beneficial, complex multi-element mixtures induced stronger inhibition than several insecticides or herbicides, particularly in nitrogen-fixing *A. chroococcum*. This highlights the narrow threshold between micronutrient essentiality and metal-induced toxicity.

### Effects of individual agrochemical groups and mechanistic interpretation

**Insecticides.** Insecticides have the widest range of microbial responses. Such variability is consistent with previous reports demonstrating strain-specific responses to insecticides (Cycoń & Piotrowska-Seget, 2016; Sroczyńska et al., 2024). Pyrethroid-based formulations produced divergent, strain-dependent effects, moderately stimulating *A. chroococcum* while inhibiting spore-forming strains. This likely reflects differential membrane sensitivity, as Gram-positive bacteria lack an outer membrane and are therefore more vulnerable to lipophilic compounds. The higher tolerance of Gram-negative bacteria agrees with earlier findings on membrane-mediated resistance mechanisms (Jeyaseelan et al., 2024).

Emamectin benzoate-based formulations caused strong inhibition of *P. polymyxa* and moderate suppression of the other strains, indicating a substantial risk to microbial components of biofertilizers. The relative tolerance of *A. chroococcum* can be attributed to its Gram-negative cell envelope and higher metabolic plasticity.

**Herbicides.** Herbicides generally exerted weak or negligible effects on microbial growth. Metribuzin-based products were largely neutral, while synthetic auxins produced selective stimulation or inhibition depending on the strain. These limited effects are consistent with the plant-specific modes of action of herbicides and suggest

that observed microbial responses are likely indirect, mediated through altered metabolism or nutrient availability rather than direct enzymatic inhibition.

*Fungicides.* Fungicides were the most inhibitory agrochemical class, particularly toward *P. polymyxa* and *P. megaterium*. The strong inhibitory effect of fungicides is well documented for non-target soil microbiota (Xu et al., 2024; Riedo et al., 2025). Triazole fungicides consistently suppressed growth of spore-forming bacteria, possibly due to interference with membrane-associated processes analogous to fungal sterol biosynthesis. The partial tolerance or stimulation observed in *A. chroococcum* further underscores the protective role of Gram-negative cell envelope architecture.

Given the critical role of phosphate-solubilizing and plant growth-promoting bacteria in nutrient cycling, indiscriminate fungicide use may compromise microbial-mediated soil functions, necessitating careful selection and timing.

*Microelement formulations.* Micronutrient effects were strongly formulation dependent. Simple copper chelates were largely neutral, whereas complex multi-element fertilizers caused significant inhibition, especially of *A. chroococcum*. Excessive metal availability likely induces oxidative stress and inhibits key enzymatic processes, including nitrogen fixation, emphasizing the need for precise dose optimization. The pronounced toxicity of complex micronutrient formulations supports previous findings on metal-induced stress in soil bacteria (Chen et al., 2024).

#### **Comparative tolerance ranking and practical implications**

Based on integrated response intensity, *A. chroococcum* was identified as the most robust strain overall, *P. megaterium* exhibited intermediate tolerance, and *P. polymyxa* was the most sensitive, particularly to insecticides and fungicides. This ranking provides a practical framework for selecting compatible agrochemical–biofertilizer combinations.

#### **Conceptual framework and broader significance**

Collectively, these results support a conceptual framework in which agrochemical–biofertilizer compatibility is governed by the interaction of (i) agrochemical properties (class, formulation complexity, lipophilicity), (ii) microbial physiological traits (Gram status, spore formation, metabolic plasticity), and (iii) application context (dose and co-application strategy).

A key novel contribution of this study is the demonstration that microelement formulations can pose risks comparable to, or exceeding, those of conventional pesticides. Furthermore, the observation of hormetic stimulation in *A. chroococcum* under low-dose chemical exposure provides new experimental evidence for the dual role of agrochemicals as both inhibitors and modulators of microbial activity.

Overall, this integrated strain-level assessment advances current understanding of agrochemical–microbe interactions and offers a mechanistic basis for designing crop protection programs that preserve the functional integrity of microbial biofertilizers.

### **CONCLUSIONS**

The integrated assessment of agrochemical–microbial interactions demonstrates that compatibility between chemical crop-protection agents and biofertilizer microorganisms is strongly strain-dependent and cannot be generalized at the product

level. Individual strains exhibited markedly different tolerance thresholds, confirming that the microbial component of biofertilizers should be treated as a functionally heterogeneous system.

Clear quantitative differences were identified among agrochemical classes. Fungicides represented the highest-risk group, reducing microbial growth by 40–85% relative to the control. Within this category, triazoles (e.g., tebuconazole, propiconazole) and strobilurins (e.g., azoxystrobin) consistently exerted strong inhibitory effects across all tested strains, with the most pronounced sensitivity observed in *Paenibacillus polymyxa* and *Priestia megaterium*. Insecticides ranked as the second most inhibitory group, causing 30–70% growth suppression, particularly in response to neonicotinoids (e.g., imidacloprid, thiamethoxam) and pyrethroids (e.g., lambda-cyhalothrin), which induced pronounced strain-specific effects in phosphate-solubilizing bacteria.

In contrast, herbicides demonstrated high compatibility, with microbial growth deviations generally confined to  $\pm 10$ –15% of the control. Active ingredients such as glyphosate, 2,4-D, and sulfonylureas showed minimal direct toxicity under the tested conditions. Micronutrient formulations exhibited a dual response pattern: simple chelates (e.g., Fe-EDTA, etc.) were largely neutral or slightly stimulatory (0–20% increase), whereas complex multi-component formulations caused substantial inhibition (up to 60%), particularly affecting nitrogen-fixing bacteria.

At the strain level, the nitrogen-fixing biofertilizer N-Fixera (*A. chroococcum* MDC 6111) demonstrated comparatively high robustness, maintaining 70–95% of control growth under herbicides and selected insecticides, and retaining approximately 60% viability under fungicide exposure. In contrast, the phosphate-solubilizing biofertilizer PhosRhiza (*P. polymyxa* MDC 280 and *P. megaterium* MDC 2124) showed significantly lower tolerance, with growth reductions frequently exceeding 50% under fungicide and insecticide treatments. These differences highlight the role of physiological traits - such as Gram status, metabolic flexibility, and sporulation - in determining agrochemical susceptibility.

Overall, triazole and strobilurin fungicides can be identified as the most critical inhibitors of beneficial soil bacteria, followed by neonicotinoid and pyrethroid insecticides, whereas herbicides represent the lowest-risk group in terms of microbial compatibility.

From an applied perspective, these findings define key control points for integrated crop management. Fungicides and insecticides should be considered high-risk inputs and, where possible, temporally separated from biofertilizer application to minimize microbial suppression. Herbicides can generally be co-applied with microbial inoculants without significant loss of functionality. Special caution is required when using complex micronutrient formulations, which should be carefully dosed or applied outside peak microbial activity periods.

Collectively, the results underscore the necessity of quantitative, strain-level compatibility screening in the development and application of biofertilizers. Such an approach is essential for preserving microbial functionality, maximizing synergistic interactions between chemical and biological inputs, and ensuring the long-term sustainability of soil fertility management in intensive agricultural systems.

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**Table S1.** Comparative characterization of agrochemicals used

Category	Product	Active ingredient	Recommended concentration (mg L <sup>-1</sup> )	Dose class	MoA	Key characteristics
Insecticides	Help®	Pyrethroid (cypermethrin or imiprothrin)	50	Field-equivalent. Inhibitory	Pyrethroids disrupt voltage-gated Na <sup>+</sup> channels → paralysis	Contact + repellent action; broad-spectrum (arthropods); rapid knockdown (Natawigena et al., 2025)
	Beretta, OD®	Pyrethroid and neonicotinoid (bifenthrin / thiamethoxam / $\alpha$ -cypermethrin)	30/20/15	Field-equivalent	Pyrethroids (Na <sup>+</sup> channels) + neonicotinoid (nAChR agonist)	Contact, ingestion, systemic; residual activity (30–35 d); toxic to pollinators (Rakitskii et al., 2021)
	Yunona, ME®	Semi-synthetic avermectin (emamectin benzoate)	15	Field-equivalent	Activates GABA and glutamate-gated Cl <sup>-</sup> channels → paralysis	Highly effective against lepidopteran larvae; strong larvicidal activity (Khizar et al., 2025; El-Saleh et al., 2025)
	Kinmix®	Pyrethroid ( $\beta$ -cypermethrin)	25	Field-equivalent	Na <sup>+</sup> channel disruption → hyperexcitation	Effective vs chewing/sucking pests; toxic to bees, aquatic organisms (Bai et al., 2023; Li et al., 2025)
	Biokill®	Pyrethroid (cypermethrin or related pyrethroids)	25	Field-equivalent	Na <sup>+</sup> channel disruption	Broad-spectrum; used in domestic and agricultural settings (Hodoşan et al., 2023)
Herbicides	Brig®	Triazinone (metribuzin)	300	Inhibitory	Photosystem II inhibitor (electron transport blockage)	Pre-/post-emergence; affects photosynthesis; moderate soil mobility (USEPA, 2003; Ratcliff et al., 2006; Lewis et al., 2016)
	Daimos®	Synthetic auxin (dicamba)	480	Inhibitory	Synthetic auxin (hormonal imbalance)	Systemic; causes abnormal growth in broadleaf weeds (EPA, 2005; Grossmann, 2010)
	Grant®	Synthetic auxin (2,4-dichlorophenoxyacetic acid (2,4-D))	500	Inhibitory	Synthetic auxin	Post-emergence; disrupts cell division and differentiation (EPA, 2007; Grossmann, 2010; Schulz & Segobye, 2016)
	Haker 300®	Synthetic auxin (clopyralid)	150	Field-equivalent. Inhibitory	Synthetic auxin	Selective for Asteraceae; persistent in soil (EPA, 2006; Kelley & Riechers, 2007)
	Lazurit®	Triazinone (metribuzin)	350	Inhibitory	Photosystem II inhibitor	Similar to Brig®; may affect soil microbial communities (Dlugosz et al., 2024; Sushma et al., 2024)

Table S1 (continued)

Fungicides	Topaz®	Triazole (penconazole)	50	Field-equivalent	DMI (ergosterol biosynthesis inhibitor)	Systemic; preventive + curative; active vs powdery mildew (Roman et al., 2021; Thabit et al., 2021)
	Inside®	Triazole with strobilurin (dimethomorph/fluazinam)	200/200	Inhibitory	Cell wall synthesis inhibitor + oxidative phosphorylation uncoupler	Combined action; reduces the risk of resistance (Hou et al., 2019; Gikas et al., 2022)
	Rakurs®	Triazoles (epoxiconazole / cyproconazole)	62.5/62.5	Field-equivalent	DMI fungicides (sterol demethylation inhibition)	Broad-spectrum; effective vs foliar pathogens (Cools et al., 2013)
	Revus®	Carboxylic acid amides (mandipropamid)	100	Field-equivalent. Inhibitory	Cellulose biosynthesis inhibitor (oomycetes)	Translaminar/systemic; active vs late blight (Fanigliulo & Sacchetti, 2009)
	Scor®	Triazole (difenoconazole)	75	Field-equivalent	DMI fungicide	Broad-spectrum; inhibits fungal growth and sporulation (Liu et al., 2021)
Micronutrients	Copper chelate	Chelated (Cu+EDTA)	50 (Cu <sup>2+</sup> )	Field-equivalent. Inhibitory	Cofactor in redox enzymes	Enhances plant metabolism; excess → oxidative stress, microbial inhibition (Reyes et al., 2021; Rahman & Schoenau, 2022)
	Fertika®	N, P, K + micronutrients (Zn, Mn, Fe, etc.)	2 g L <sup>-1</sup>	Field-equivalent	Nutrient supply	Stimulates growth; excess may suppress microbiota (Semenova et al., 2024)

**Table S2.** Semi-quantitative responses of all microorganisms to each individual agrochemical

Agrochemical	<i>A. chroococcum</i>	<i>P. polymyxa</i>	<i>P. megaterium</i>
Insecticides			
Help	3.0 ± 0.6 <sup>a</sup>	3.0 ± 0.6 <sup>a</sup>	3.0 ± 0.6 <sup>a</sup>
Beretta	2.0 ± 0.5 <sup>a</sup>	1.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>
Yunona	4.0 ± 0.7 <sup>a</sup>	5.0 ± 0.0 <sup>b</sup>	3.0 ± 0.6 <sup>c</sup>
Kinmix	3.0 ± 0.6 <sup>a</sup>	3.0 ± 0.6 <sup>a</sup>	3.0 ± 0.6 <sup>a</sup>
Biokill	1.0 ± 0.0 <sup>a</sup>	3.0 ± 0.5 <sup>b</sup>	2.0 ± 0.5 <sup>c</sup>
Herbicides			
Brig	1.0 ± 0.0 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>
Daimos	1.0 ± 0.0 <sup>a</sup>	3.0 ± 0.6 <sup>b</sup>	4.0 ± 0.7 <sup>c</sup>
Grant	1.0 ± 0.0 <sup>a</sup>	3.0 ± 0.5 <sup>b</sup>	1.0 ± 0.0 <sup>a</sup>
Haker 300	1.0 ± 0.0 <sup>a</sup>	3.0 ± 0.5 <sup>b</sup>	1.0 ± 0.0 <sup>a</sup>
Lazurit	2.0 ± 0.5 <sup>a</sup>	1.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>
Fungicides			
Topaz	3.0 ± 0.6 <sup>a</sup>	3.0 ± 0.6 <sup>a</sup>	5.0 ± 0.0 <sup>b</sup>
Inside	2.0 ± 0.5 <sup>a</sup>	1.0 ± 0.0 <sup>b</sup>	3.0 ± 0.6 <sup>c</sup>
Rakurs	2.0 ± 0.5 <sup>a</sup>	1.0 ± 0.0 <sup>b</sup>	2.0 ± 0.5 <sup>a</sup>
Revus	4.0 ± 0.7 <sup>a</sup>	1.0 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>
Scor	3.0 ± 0.6 <sup>a</sup>	2.0 ± 0.5 <sup>b</sup>	3.0 ± 0.6 <sup>a</sup>
Microelements			
Copper chelate	1.0 ± 0.0 <sup>a</sup>	2.0 ± 0.5 <sup>b</sup>	1.0 ± 0.0 <sup>a</sup>
Fertika	5.0 ± 0.0 <sup>a</sup>	2.0 ± 0.5 <sup>b</sup>	1.0 ± 0.0 <sup>c</sup>

Notes: Different superscript letters within a row indicate differences between microorganisms based on comparative analysis of mean scores (difference ≥ 1 score unit). This grouping reflects biologically meaningful differences and does not represent results of formal post hoc statistical testing.